(2)

Loma Linda University

A Seventh-day Adventist Institution

School of Medicine
Department of Medicine

April 12, 1989

Dr. Jeannine A. Majde
Program Manager, Biology Systems
Office of Naval Research
Physiology Branch, Code 441
800 North Quincy Street, Room 443
Arington, VA 22217

Re: ONR Contract N00014-84-K-0393

Dear Dr. Majde:

My colleagues and I submit for your consideration a final report on the above contract, entitled "Cellular and Organismal Responses to Combined Kilohertz and Other Nonionizing Electromagnetic Fields".

As part of this document, you will find a copy of the early final report submitted to Dr. Thomas Rozzeli summarizing work done under the original two years contract (4-29-84 to 4-28-86). This document, that also included a request for continuation of funding, was not mailed to members of the Bioelectromagnetics Program in the Distribution list. Only three copies without the Report Documentation Page were sent at that time, as explained on the cover letter.

Since funding for the additional year (4-29-86 to 4-28-87) was granted, we complied with regulations by submission of an Annual (7-29-86) and a Semi-Annual (12-9-86) reports. In response to the latter, we received an ONR letter (copy attached) indicating that our report requirements under the terms of this contract have been fulfilled. However, an official final report is still missing from our files at your agency. To put an end to this disparity, we are now enclosing a report of all research activities developed during this period.

Questions may be directed to my office (714)825-7084 x2264 during normal business hours.

W. ROSS ADEY, M.D.
Principal Investigator

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Cellular and Organizational Responses to Combined Kilohertz and Other Nonionizing EM Fields

12. PERSONAL AUTHOR(S)
W. Ross Adey, M.D.(PI), S.Bawin, C.Cain, A.Sheppard, M.Stell, B.Vasquez, Ph.Ds (Co-PIs)

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The three elements of the research program were designed to examine possible biological and medical effects of exposure to the near-field of Loran-C transmitters that operates in a pulse modulated mode at a carrier frequency of 100 KHz.

1. Neurochemical studies tested the effects of single and repeated in vivo exposure to a simulated Loran-C type waveform on the rat brain biogenic amine and opiate receptor systems. We found AM-PM differences in the levels of amines but no field-related effect was detected. Number and affinity of opiate receptors were also not affected by exposure to the combined electric (3 kV/m rms) and magnetic (48 uT rms) components of this Loran-C type waveform.

2. Behavioral studies compared the rat's known ability to detect low intensity 60 Hz electric fields (<10 kV/m) with potential detection of Loran-like fields. The data showed that rats trained in an operant conditioning task did not reliably detect the 100 KHz at any field strength used (0 to 9 kV/m). (See back)

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JEANNINE A. MAJDE, Ph.D	(202) 696-4053	ONR

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3. Biochemical studies measured ornithine decarboxylase (ODC) activity in tissue culture cells to monitor membrane signal transduction. Exposure to the Loran-C type waveform at 10mV/cm field strength did not modify basal ODC activity in primary bone cells from mouse calvaria. (AU)

DEPARTMENT OF THE NAVY
OFFICE OF THE CHIEF OF NAVAL RESEARCH
ARLINGTON, VIRGINIA 22217-5000

IN REPLY REFER TO
5000
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24 Dec 86

Beatriz J. Vasquez, Ph.D.
Jerry L. Pettis Memorial
Veterans' Hospital
11201 Benton Street
Loma Linda, CA 92357

Dear Dr. Vasquez:

Thank you for sending us your Semiannual Report, entitled "Cellular and Organismal Responses to Combined Kilohertz and other Nonionizing Electromagnetic Fields" under your Office of Naval Research Contract N00014-84-K-0393.

This report fulfills the report requirements under the terms of this contract. Thank you for participating in our research program and good luck to you in your future work.

Sincerely yours,

JEANNINE A. MAJDE, Ph.D.
Program Manager
Systems Biology

Copy to:
Dr. Rafferty
ONR Resident Representative, Pasadena, CA

Jerry L. Pettis Memorial
Veterans' Hospital

11201 Benton Street
Loma Linda CA 92357

Veterans Administration

January 14, 1986

In Reply Refer To:
605/151

Dr. Thomas Rozzell
Office of Naval Research
Physiology Branch, Code 441
800 North Qunincy Street, Room 443
Arlington, VA 22217

Re: ONR Contract N00014-84-K-0393

Dear Dr. Rozzell:

My colleagues and I submit for your consideration a final report on the above contract, entitled "Cellular and Organismal Responses to Combined Kilohertz and Other Nonionizing Electromagnetic Fields."

We also submit a request for a continuation of funding for one year, for the period April 29, 1986 to April 28, 1987.

We are pleased to report that the exposure facility which we have designed and constructed to simulate fields associated with Loran C transmitter sites is in full operation and that data collection in the three assigned areas of the contract is on schedule.

We enclose three copies for your review. If additional copies are needed, would you please let me or Dr. Vasquez know.

We deeply appreciate the continuing interest of ONR in these studies.

Sincerely,

W. Ross Adey, M.D.
Associate Chief of Staff for Research
and Development

Contract Proposal:

CELLULAR AND ORGANISMAL RESPONSES TO COMBINED KILOHERTZ AND OTHER NONIONIZING ELECTROMAGNETIC FIELDS

Submitted to:

Office of Naval Research
Physiology Branch, Code 441
Attention: Dr. Thomas Rozzell
800 North Quincy Street, Room 443
Arlington VA 22217

Principal Investigator:

W.R. Adey, M.D.
Associate Chief of Staff for Research and Development
Jerry L. Pettis Memorial Veterans Hospital
11201 Benton Street
Loma Linda CA 92357
Phone: (714) 825-7084, ext. 2264; FTS 996-2264

Co-Investigators:

S.M Bawin, Ph.D.
C.D. Cain, Ph.D.
A.R. Sheppard, Ph.D.
M.E. Stell, Ph.D.
B.J. Vasquez, Ph.D.

Project Period: April 29, 1986, to April 28, 1987

Officer Authorized to Sign for Institution:

Mr. John Richards, Director
Grants Management Office
Loma Linda University
Loma Linda CA 92350
Phone: (714) 796-3741, ext 4398

Date Submitted: January 14, 1986

Signature of Principal Investigator: W R Adey Date: January 14 1986

Table of Contents

SUMMARY

1. Navy communication systems make extensive use of high-powered, low frequency transmitters operating in the 10 - 100 kHz range. These systems utilize a variety of modulation systems, including FSK or MSK methods for teletype and pulsed modulation methods for radiolocation (eg. Loran C).

2. Fields in the vicinity of antennas and tuned transmission lines at these transmitter locations are large. For Loran C transmitters electric fields may be as high as 2500 V/m and magnetic fields up to 200 A/m.

3. We have constructed an exposure facility suitable for testing small animals and other biota. The system operates at 100 kHz and the pattern of pulse modulation simulates the Loran C technique (a sequence of pulses (8 on slave transmitters, 9 on master transmitters), pulse duration 1.0 msec, interpulse inverval 1.5 msec, pulse burst repetition rate 16/sec). The signal (less than 10 W) is delivered to a parallel-tuned tank circuit, with an inductance 0.77m in diameter and 0.9m long. Experimental animals in plastic containers are exposed by placing them in the interior of the inductance.

4. Three projects have been initiated as a first approach to possible biological effects of these fields:
   a. Behavioral studies designed to test field perception by exposed animals.
   b. Examination of regional brain amine levels following field exposure.
   c. Studies of field effects on cell surface patterns of glycoprotein receptors.

5. Pilot studies in rats have disclosed no evidence of field perception at intensities up to 25 kV/m.

6. Baseline studies of ELF (60Hz) field effects on brain amine levels have examined norepinephrine, dopamine and 5-hydroxytryptamine levels and levels of their metabolites in separate analyses of tissue from striatum, hypothalamus and cortex. Norepinephrine levels in hypothalamus were responsive to 60Hz field exposures. A circadian rhythm was found in these measures, and this effect has been incorporated into experimental protocols for Loran C field exposures now in progress.

7. Baseline studies of cell surface receptor mosaic manipulation by pulsed fields at frequencies from 1 Hz to 15 kHz have shown that interpulse intervals and pulse duration are key factors determining rates of receptor displacement.

U.S. NAVY

OFFICE OF NAVAL RESEARCH

Budget

April 1986 - April 1987

Personnel

Name	Level	% Effort	Salary Amount
W. Ross Adey, M.D. Principal Investigator	-	5	-0-
S. Bawin, Ph.D.	GS-13/3	10	-0-
A. Sheppard, Ph.D.	GS-13/3	10	-0-
M. Stell, Ph.D.	GS-11/2	10	-0-
B. Vasquez, Ph.D.	Res. Chemist/LLU	85	31,852
C. Cain, Ph.D.	GS-11/2	10	-0-
R. Jones	Electronic Tech.	10	-0-
G. Bryan	GS-9/1	10	-0-
M. Malto	LLU	15	3,330
R. Helm	LLU	50	10,649
M. Mahoney	LLU	50	12,656
A. Goodwin	LLU	50	8,205
		SUBTOTAL	66,692
		FRINGE @ 25%	16,673
		TOTAL	83,365

Equipment 0

Supplies & Services

Animals		
Rats Purchase (300 @ 5.50 per)		1,653
Per Diem (17 X $.76 X 365)		4,715
Neuroblastoma Cell Line		100
Laboratory Ware		5,000
Data Acquisition		2,000
Electronic Components		2,500
Publication and Copy Service		667
	Sub Total	16,635
	Total Direct Costs	100,000
	Indirect Costs (@ 33% of Modified TDC)	-0-
	Grant Total	100,000

CURRICULUM VITAE

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EDUCATION:

Sept. 1971 - June 1975	Knox College Galesburg, Ill. 60540 B.A. Chemistry
Sept. 1977 - March 1979	Rush University Chicago, Ill. Biochemistry, Dept.
Sept. 1979 - Jan. 1986	Univ. of California, Riverside Riverside, CA. 92521 Biochemistry Dept. Ph.D., Biochemistry

EMPLOYMENT: Research Biologist, Jerry L. Pettis Memorial Hospital, Loma Linda, CA. Sept. 1985 - present

Teaching Assistant, Endocrinology, Univ. California, Riverside Biochemistry, Dept. Sept, 1981 - June 1982

Research Assistant, Biochemistry Dept., Univ. California, Riverside
Sept. 1979 - Aug. 1985

Research Assistant, Biochemistry Dept. Rush University, Chicago.
Jan. 1978 - Dec 1978

Business computer salesman, Burroughs Corp., New York, New York
Aug 1975 - April 1977

Publications:

Cain, C.D., Donato, N.J., Byus, C.B., Adey, W.R., and Luben, R.A, 1985. Pulsed electromagnetic field modifies cAMP metabolism and ornithine decarboxylase cactivity in primary bone cells. In: International Conference on electric and magnetic fields in medicine and biology. Published by Institute of Electrical Engineers, London and New York. Conference Publication number 257, pp 9-13 .

Cain, C.D., Luben, R.A., 1985. Pulsed electromagnetic field effects on PTH-stimulated cAMP acumulation and bone resorption in mouse calvaria. In: Proceedings of the 23rd Hanford Life Sciences Symposium (in press).

Luben, R.A., Cain, C.D., 1984. Use of bone cell hormone response systems to investigate bioelectromagnetic effects on membranes "in vitro". In: Adey,W.R., Lawrence, A.F. (eds), Non-linear Electrodynamics of Biological Systems. Plenum Press, New York, p.23.

Luben, R.A., Cain, C.D., Chen, M.C.Y., Rosen, D.M., and Adey, W.R. Effects of electromagnetic stimuli on bone and bone cells in vitro: Inhibition of responses to parathyroid hormone by low-energy, low-frequency fields. Proc. Natl. Acad. Sci. USA 79:4180-4184, 1982.

Abstracts:

Cain, C.D., Donato, N.J., Byus, C.V., Adey, W.R., and Luben, R.A. Pulsed electromagnetic field influences PTH-stimulated cAMP accumulation and ornithine decarboxylase activity in primary bone cells. DOE, EPRI, NYSHD Contractor's review. Washington, D.C. 1985. (abstract).

Cain, C.D., Luben, R.A., and Adey, W.R. Pulsed electromagnetic field effects on PTH stimulated cAMP accumulation and bone resorption in mouse calvariae. DOE, EPRI, NYSHD Contractor's Review, Saint Louis, Mo. 1984. (abstract)

Lin-Liu, S., Cain, C., and Adey, W.R. Effects of ELF on forskolin activation of adenylate cyclase in hippocampal slices. Bioelectromagnetic Soc., 6th Annual Meeting, Atlanta, GA, July 15, 1984. Proceedings, p. 5.

Lin-Liu, S., Cain, C.D., and Adey, W.R. Effects of ELF fields on forskolin-induced cAMP accumulation in hippocampal slices. DOE, EPRI, NYSHD Contractor's Review, Saint Louis, Mo. 1984. (abstract).

Cain, C.D., Luben, R.A., Time dependant aspects of pulsed electromagnetic field effects on bone metabolism. DOE, EPRI contractor's review. Kansas City, Mo. 1983. (abstract)

1.0 INTRODUCTION

1.1 RESEARCH PLANS

Initial program goals identified three areas of study of possible interactions with LF fields in the kHz range: behavioral effects in animal models: neurochemical effects on catechol amine levels in specific brain nuclei; and kinetic effects on cell surface macromolecular receptor sites.

At the same time, it was recognized that a major effort would be devoted to development and calibration of appropriate exposure facilities that would simulate all essential parameters of a selected Navy communication system.

To maximize our productivity, certain pilot studies have been conducted at ELF frequencies during development of an exposure facility with the required field parameters. These studies have provided valuable baseline data for comparison with those now being acquired in exposures in the 100 kHz facility.

In the research protocol now submitted, we plan the following areas of principal effort that will carry the contractual commitment to completion:

1. Behavioral studies in a rat model will use tracking techniques to termine whether the subjects may detect the presence of the field In the light of findings in a pilot study, attention will be directed to the significance of field strength and of field duty cycles, and to modification of initial training procedures to maximize sensitivity of the experimental method.

2. Studies of field effects on brain amine levels will pay special attention to circadian detected in pilot studies. These circadian effects were clearly sufficient to mask possible responses to field exposure.

3. Studies of cell surface receptor mechanisms will extend pilot studies that have examined a broad spectrum of pulsed fields at frequencies from 1Hz to 15 kHz. Cell cultures will be exposed to the 100 kHz simulated Loran C field developed for this project.

1.2 RATIONALE

Pilot studies in two animals discussed below have not revealed behavioral sensitivities to the simulated Loran C field. The strength of the field may be insufficient. However, the exposures have been conducted at field levels as high as 25 kV/m, a level substantially in excess of those reported at Loran C sites. It is also possible that the duty cycle in this field may be too short for optimal detection. Nevertheless, it is not proposed to move beyond available field parameters until different behavioral training and testing options discussed below have been fully evaluated.

Pilot studies of brain amine levels and their sensitivities to 60 Hz

field exposures have revealed differential regional effects that suggest the importance of a similar approach in Loran C field exposures. Also, strong evidence provided by these pilot studies for circadian rhythm effects clearly mandate a rigorous experimental protocol in sampling procedures for the Loran C studies.

The kinetics of cell surface receptor mobility for myoblasts as models of excitable cells have been specified in studies covering frequencies as high as 15 kHz. This detailed baseline will be valuable in evaluating possible effects in exposures to the Loran C field. Aspects of physicalchemical interactions at cell membrane surfaces sensitive to pericellular EM fields that involve surface fixed charged characteristics are now being investigated by Dr. C.D. Cain, (CV included), complement those of Dr. S. Lin-Liu, whose current activities have been curtailed by reason of other commitments.

2.0 ELECTROMAGNETIC FIELD PARAMETERS AND INSTRUMENTATION.

A. R. Sheppard, R.A. Jones, M. E. Stell, W.R. Adey.

2.1 BIOLOGICAL AND ENVIRONMENTAL DESIGN CONSIDERATIONS

This study explores the possibility of biological interactions in a frequency range for which there is little laboratory research. It is prompted by issues of environmental health attending particular radio transmitters operating at frequencies of 10 to 100 kHz. These include the Loran-C System that operates with a carrier frequency of 100 kHz with modulation sidebands of $\pm$15 kHz. In view of the wide range of environmental conditions, there was considerable uncertainty in making the choice of exposure characteristics that would be 1) satisfactory simulations of the field characteristics encountered in the environment of these transmitter sites, and 2) would also be satisfactory for biological experimentation. Specific problems entering into the experimental design include coupling of the field to the test system and determination of field conditions of greatest biological sensitivity.

2.2 RATIONALE FOR CHOICE OF WAVEFORM CHARACTERISTICS

Following consultation with ONR (Marron, 1984,pers. comm.) the decision was made to focus on the Loran-C waveform which involves a pulse-modulated carrier at 100 KHz. Other environmental radio emissions in the kilohertz range include the Omega System at 10 to 14 kHz. Dr. Richard Tell of the EPA, Las Vegas (1985, pers. comm.) kindly provided information on the characteristics of the Loran-C transmitter system including a description of the master and slave station pulse modulation schemes. Each chain of Loran C stations includes a master station that transmits 9 pulses per burst and slaves that transmit 8 pulses per burst at fixed intervals after the master station's ninth pulse. For example,the west coast chain involves a master at Fallon, NV and slave stations at George, WA, Middletown, CA and Searchlight, NV. The eight-pulse bursts of each slave are identical, but each begins at a specific interval following the first pulse of the master station. For the slave stations listed above, these times are 11.000, 27.000 and 40.000 ms. At 99.400 ms from the initial pulse, the cycle re-starts. Geographic position is triangulated by noting the change in these relative times due to travel time delays in these signals.

The transmitted pulses have an envelope given by the function, $A(t) = A_0\ t^2\ \exp(-2t/65)$, where t is in microseconds and A_0 is the amplitude. This function, as shown in Fig. 1, produces a "teardrop" shaped pulse envelope. The interval between pulses is 1.000 ms, except for the master station's ninth pulse which occurs 2.000 ms following the 8th pulse.

The variable aspects of the Loran-C signal principally involve the inclusion of the ninth pulse for master stations, chain-determined variations in the intervals between pulse bursts, and chain-dependent variations in the phase angle (0 or 180 degrees) at the initial zero-crossing of the signal within the pulse envelope. The emissions occupy a bandwith in excess of

20kHz at 100 kHz. At present there is no reason to believe that any of the waveform details is significant biologically. The major features of the frequency spectrum are determined by the pulse shape, the 1.000 ms interpulse interval, the 8.000 ms (or 10.000 ms for the master station) burst duration, the 100 kHz carrier and the approximately 100 ms repetition interval. The differences in the spectral character of the various waveforms follow from the 10 ms burst duration of a master station and the variable repetition rate for each chain of stations.

In addition there are minor changes made when certain pulses are "blinked" on or off to encode operational information (such as a transmitter problem). Master stations blink the ninth pulse for 200-250 ms or for 750-800 ms, while secondary stations blink the first and second pulses "on" for durations of between 200 and 350 ms with a four second periodicity for repetition of the blink. These blink modulations thus introduce frequency components with fundamentals related to the aforementioned periodicities.

2.2.1 Description of the Simulated Loran C Waveform

The simulated waveform is a pulse-modulated 100 kHz carrier with 9 pulses per burst. The interval between pulses is 1.500 ms, the interval between bursts is 40.000 ms. The pulse envelope is approximately square with a duration of 1.000 ms. Pulse rise times of 40 to 200 us, are determined by power amplifier and load considerations for each apparatus. Table 1 summarizes these parameters in comparison with actual Loran C waveforms. Except for the pulse risetime and envelope shape, the timing parameters are easily adjusted by changing the microcode in the pulse generator described below. The risetime is determined by the amplifier load characteristics. The simulated waveform does not attempt to recreate the actual pulse envelope.

Principal differences between the actual Loran C waveform and the laboratory simulation are in the interpulse interval (1.000 vs. 1.500 ms), pulse shape ("teardrop" vs. square), pulse duration (about 250 vs. 1000 us) and in the absence of a doubly-long delay prior to the ninth pulse. These differences result in spectral differences (see below).

2.2.2 Spectral Characteristics of the Waveform

We have not yet obtained direct spectra of the waveform, but intend to do so upon obtaining the necessary equipment. From examination of the waveform we have chosen we expect the energy to be localized to the following frequency bands:

a) a sub-ELF band signal determined by the pulse repetition rate of 16 Hz (40 ms interburst interval plus 21 ms burst duration).

b) a VF band signal at 400 Hz determined by the 2.5 ms period between pulses within a burst (1 ms pulse duration plus 1.5 ms interpulse interval).

c) a LF band signal at 100 kHz determined by the sinusoidal carrier

frequency.

d) a VLF band signal related to side bands in the range 5 to 25 kHz produced by the finite pulse risetime that lies in the range 40 to 200 us.

2.3 DESIGN OF EXPOSURE APPARATUS

All apparatus were custom-designed in our laboratory to meet the foregoing criteria. Translation of those criteria into practical apparatus led to the following engineering designs:

a) Behavioral studies: Provide a parallel plate capacitor to create a homogeneous E-field at 100 kHz over the volume of an acrylic rat housing. Provide electric field shielding to reduce environmental fields outside the test chamber. The housing contains optically-coupled response levers and response indicators for use in the behavioral testing. The E-field levels, behavioral stimuli, and animal responses are all under computer control from the Digital Equipment Corp. PDP 11/70.

b) Neurochemical studies: Provide a solenoidally wound coil of wire with end-electrodes. Provide a drive amplifier (ENI 2100L), step-up transformer (ENI, custom manufacture), and a variable vacuum capacitor (Jennings UCSXF-1200) to energize the solenoid in parallel resonance at 100 kHz. The interior volume of the coil is designed to produce uniform fields over the volume occupied by the rats. Animals are housed in acrylic cages that permit adequate ventilation and light. Access to the cages is to be provided for regular maintenance and for cleaning and exchanging of cages.

c) In vitro studies: Provide a constant current amplifier operating at 100 kHz and without distortion of the pulse envelope with sufficient voltage to operate a series-wired set of exposure troughs. Each trough is coupled to the current source via an agar bridge of sufficient cross section to reduce local current densities to values near the mean current density.

2.3.1 Waveform Generator

Fig. 2 shows the logical diagram for the program that operates the 8085 microprocessor incorporated in a Pro-Log 7805 computer board. Fig. 3 is a schematic representation of the output waveform. A model 8085 8-bit microprocessor was chosen to permit flexibility in pulse parameter generation and economical software production. Once debugged, the microcode is programmed into the erasable memory (EPROM).

2.4 ELECTRIC AND MAGNETIC FIELD PARAMETERS

Our aim is to duplicate the high E- (and H-) field intensities associated with the tuned feedlines used with LF transmitters or fields near the antenna tower base. Surveys of Loran-C sites (McEnroe, 1980) indicated typical E-field strengths of 200 V/m square root of the sum of squares (rss) or 50 V/m square root of the mean squared (rms). The maximum values were

much greater, 2.7 kV/m rss, 0.6 kV/m rms. The same survey indicated magnetic field strengths were typically 0.1 uT rss, 0.1 uT rms and at maximum, 33 uT rss.

2.4.1 Rationale

The electric and magnetic field parameters to be used in the biological studies vary according to the experimental techniques and goals. The behavioral studies will be done using exposure to electric fields only. To provide a greater probability of a positive outcome (determination of a threshold for detection by rats) the sensory detection behavioral tests require electric fields that are numerically stronger than the kHz fields found in the environment.

The neurochemical studies will be done using exposure to combined electric and magnetic fields. Rats will be exposed repeatedly for 1 h/day, 5 d/week over a 4 week period. An apparatus to create magnetic fields provides substantial design problems. The magnetic field generator for large volumes of space can be costly because such designs can requires current and power levels that approach the scale of the power needs for the transmitters themselves. Dr. George Kamin (who has since left the laboratory) initiated a design for a solenoidal exposure device in which the electric and magnetic fields are co-generated by a solenoid operated at its resonant frequency (where the voltage across the coil length is a maximum and the out-of-phase current in the wire is also large). The engineering of this device required considerable development time beyond that needed for the parallel plate apparatus, but provides a system that better simulates the environmental conditions. The ratio of electric to magnetic fields is representative of the ratio of the maximum electric to the maximum magnetic field at a transmitter site, but is not necessarily representative of the actual ratio for any particular site of an antenna or transmitter facility.

The *in vitro* exposure apparatus is capable of producing electric field strengths of the magnitude known to be biologically significant at lower frequencies (0 to 60 Hz) but weaker than the field strengths at which Pickard and co-workers (Pickard and Rosenbaum, 1978, Pickard and Barsoum, 1981, Barsoum and Pickard, 1982) observed membrane rectification in the range 25 kHz to 10 MHz. Levels of field strength applied to the cells in culture can be scaled to lower values that may be calculated to represent a specific in situ exposure to body tissues.

2.4.2 Electric Field Magnitude

As shown in Table 1, the laboratory apparatus produces E-field strengths that are greater than those found at the transmitter sites. In the parallel plate exposure system (for the behavioral study) field strengths of 9.0 kV/m rms are possible. In the solenoidal apparatus (neurochemical study) the maximum is about 3.3 kV/m rms. For the *in vitro* study, tissue-level field strengths of 10 mV/cm will be obtained.

2.4.3 Magnetic Field Magnitude

The solenoidal apparatus has been designed to produce 85 uT (67 A/m, or 0.85 G) rms. Both the parallel plate and in vitro exposure devices produce essentially no magnetic field.

2.4.4 Electric and Magnetic Field Spatial Polarization.

For the behavioral tests, the electric field is oriented in the vertical plane, perpendicular to the rat spine during it normal posture. This is the same field orientation in which rats were tested at 60 Hz and may be related to antenna site conditions in which a person is exposed to a horizontal electric field. The solenoidal apparatus produces a magnetic field that is approximately parallel to the electric field within the solenoid. Both fields are vertical and perpendicular to the normal rat spinal axis. Because the coil is operated at resonance, the E and H fields are in opposite phase. The electric field in the culture medium is oriented parallel to the liquid surface.

2.5 FIELD MEASUREMENT TECHNIQUES

Most commercially available apparatus do not permit E- and H-field measurements in air at 100 kHz and no available instruments were suitable for measurements over the frequency and amplitude ranges used for the animal exposure devices of this study. Thus, we have started on the design and construction of suitable instruments for field calibration and mapping of field uniformity within the exposure apparatus.

The carrier frequency is determined by setting a rotary dial on the Wavetek #188 oscillator and calibrated by a Fluke #1925A frequency meter. Timing accuracy and stability of the microprocessor-generated pulse envelope is based on the integral quartz-based clock and measured using a Tektronix #465 oscilloscope with a calibrated sweep generator module.

Electric field strengths in the behavioral apparatus will be checked by measurement of the voltage on the electrode plates using a pair of Tektronix #P6015 high voltage probes and with knowledge of the plate separation. For the solenoidal apparatus, the same probes can be used as a monitor of applied voltage and to estimate E-field strengths on the basis of the distance between end-plates. Waveforms are observable on the oscilloscope.

The actual output of the electric field generation equipment is shown in figures 4a-d. These figures represent photographs of the oscilloscope screen using high voltage probes on the plates. Different sweep speeds were used to illustrated different aspects of the waveform. In Fig. 4a the sweep speed is 10 ms per division. This photo shows the pulse modulation pattern of nine individual "bursts" of carrier. In Fig. 4b the sweep speed is 0.5 ms per division and the shape of two bursts of the 100 kHz carrier are shown. In Fig. 4c the sweep speed is 10 microseconds per division and the carrier is illustrated. Note the relatively smooth rise of the waveform from zero to

maximum in about 50 microseconds. In Fig. 4d the fall off of the waveform is seen at 10 microseconds per division. Again note the relatively smooth fall off which takes about 50 microseconds.

Electric field magitudes will be directly measured using a small probe that is under development. This probe is based on the principles developed by Misakian (1978) at the National Bureau of Standards. The hemispherical aluminum probe shells will contain electronics that will be operated by an on-board battery. The data will be sent by an infra-red signal through a fiber optic guide to a remote phototransistor located outside the field region.

Magnetic field strengths can be measured now by a low-capacitance, low inductance search coil connected by magnetically shielded wire to a remote high impedance voltmeter. The search coil is calibrated in a Helmholtz Coil which in turn is calibrated by a Hall Effect Probe (Bell # FTB 0415 and Bell 610 gaussmeter). Frequency-dependent effects in the performance of the calibration instruments and coils, including unintended resonance conditions were carefully examined to ensure accurate extrapolation from the DC calibration standard.

Electric field strength in the in vitro apparatus is measured directly by a pair of electrodes in a medium of reduced conductivity. These values are then used to calibrate the electric field strength in terms of total current in the dishes. In the usual experimental conditions the total current and depth of the physiological medium (i.e., area through which the current flows) are monitored.

2.6 CONSTRUCTION OF EXPOSURE APPARATUS

2.6.1 Whole Animal Exposure for Behavioral Studies

Figure 5 (diagram) and Fig. 6 (photo) illustrate the apparatus used for behavioral studies. It resulted from a modification of an existing design used for the 60 Hz studies. A pair of square aluminum plates, 0.33 x 0,33m with a welded guard-ring along the periphery, have a separation of 0.33m. Within this gap a behavioral test chamber constructed entirely of acrylic plastic is placed within a few cm of the bottom electrode. De-ionized (low-conductivity) water is delivered in a vinyl tube to the animal by a remote solenoid valve operated by the computer. Behavioral levers and lights (visual stimuli) are provided by glass fibers that originate in computer--controlled light sources or light sensors. The high voltage amplifier and transformer are located outside the chamber and connected to the plates by coaxial lines. The electrode assembly and behavioral testing device are contained within a pressed-wood cabinet that provides sound, light and olfactory isolation from the environment and provides a constant background illumination and white noise. The interior surface of the cabinet is covered with grounded copper foil to provide electromagnetic shielding.

Ambient lighting is provided by electrically shielded bulbs near the upper plate. An electrically shielded speaker is also provided for production of a constant 65 dB-SPL background white noise. A fan is used to provide a constant turnover of air within the isolation box that houses the test chamber. Previous experience has shown that the cages must be carefully cleaned and dried at regular intervals to avoid shunting of the field by test chamber wall conduction. Test chambers are disassembled and cleaned once every two to three days, and then hand dried.

Output of the press panel fiber optic guides is fed to a custom built interface. The interface connects to a Digital Equipment Corp. PDP-11/70 computer. The computer controls the illumination spot on each press panel, the solenoid valve that causes the subjects to receive their water reinforcement, and the electric field generation equipment. During the experiment all operations, e.g. the subject's presses on the panels, the computer's reinforcement delivery, field level, etc., are recorded on disk. After the experiment all individual items are analyzed and summaries are stored in a data base built by the experimental software. Analysis of these data are done using Digital Equipment Corp.'s data base inquiry system, "Datatrieve", thus allowing extreme flexibility of data analysis.

2.6.2 Whole Animal Exposure for Neurochemical Analyses

Figure 7 (diagram) and Fig. 8 (photo) illustrate the apparatus for the neurochemical studies. The solenoidal exposure apparatus is constructed of about 500m of #15 enameled copper wire wound on the outer surface of an opaque polystyrene sheet rolled into a cylinder and braced internally by a pair of wooden rims and four longitudinal stringers. Wood parts were held together by epoxy resin cement and sealed against moisture by a coat of a polyurethane varnish (Deftane). The length and diameter of the coil are 0.9m and 0.77m, respectively. The coil is held at 0.255m above the surface of the floor by a wooden stand. The electrical resistance of the coil is 5.2 ohms and the inductance 21 millihenries.

The two electric field "plates" at each of the cylinder ends are spoked metal rods which are each electrically connected to the adjacent coil end. Connection to the step-up transformer located in a nearby equipment rack is by coaxial cable. The entire apparatus is contained in a cubical shielded room 2.06 m on each side and constructed of wood framing to which hardware cloth was stapled. Two identical shielded enclosures and solenoids were constructed and either may be selected as the sham or test chamber by switching the cables located in the equipment rack between the two enclosures.

The enclosures are located in a well ventilated room in the vivarium facilities. Temperature and humidity levels are stable as a result of regulation by the hospital environmental control system. Except during exposure periods, animals will be housed in a nearby room under the same lnvironmental conditions. Light is on a 12:12 cycle (lights on at 0600h).

2.6.3 <u>In Vitro</u> Exposure of Cultured Tissue

Figure 9 shows the apparatus schematically. The in vitro apparatus will consist of standard square plastic petri dishes connected by agar bridges. The bridges are made of hemi-cylinders obtained by slicing sections of glass tubing in half along their lengths. The volume to be filled with agar is formed by joining (with glass) the top and bottom ends of two pieces of different diameter. These bridges have a slot-like cross-sectional area that is formed by the space between the two hemi-cylinders of different diameters. Current is passed in series through the test dishes. A source of constant current at 100 kHz will be developed by modification of existing operational amplifier designs used at ELF frequencies. The entire set of dishes is kept at constant temperature and humidity in a standard laboratory incubator that also enables control of the atmosphere to 5% CO2/95 % air.

2.7 PERSONNEL SAFETY

Personnel safety near the behavioral chambers is provided by an independent circuit that interrupts the input to the power amplifier if an overvoltage condition is detected, if the chamber door is opened, or if the experimental protocol, i.e. the experimental software, calls for no stimulus to be present. Access to the room is limited by locked doors.

Personnel safety near the solenoidal chambers is ensured by interlocks on the enclosure doors, by signs warning of the hazardous voltages present, and by limiting access to the room (locked room doors). An independent safety circuit shuts down the high voltage supply if an output overvoltage condition is detected, or if the chamber door is opened.

Total current that can be delivered at high voltage by either of the apparatus is unlikely to be lethal, but could produce severely painful rf burns in localized, but deep, tissues. The in vitro apparatus poses no special safety hazards.

2.8 EXPERIMENTAL INTEGRITY

For the behavioral experiment daily assesment of the subjects' apparent health and their weight are made. The behavior of the subjects is analyzed weekly to determine if it deviates substantially from that expected. Experimental data are backed up on duplicate magnetic tapes on a weekly basis to minimize any potential data losses. Checks for proper operation of the field generation apparatus are made daily and monthly. A peak reading voltmeter is built into the field generation system. The meters are calibrated to read kV/m (rms) for the unmodulated portion of the carrier directly. This system is checked daily. Once a month the high voltage probes and an oscilloscope are used to read the plate voltage directly for independent confirmation of the actual plate voltage.

For the solenoidal apparatus the field waveform will be examined at weekly intervals in order to guard against undetected changes in amplifier performance or other causes of altered exposure conditions

For the in vitro system currents will be measured daily with ammeters that are an integral part of the apparatus and waveforms will be checked by oscilloscope periodically. The depth of medium in the dishes is kept constant by carefully pipetting all solutions with volumetric pipets and by keeping the dishes covered and at 100% humidity.

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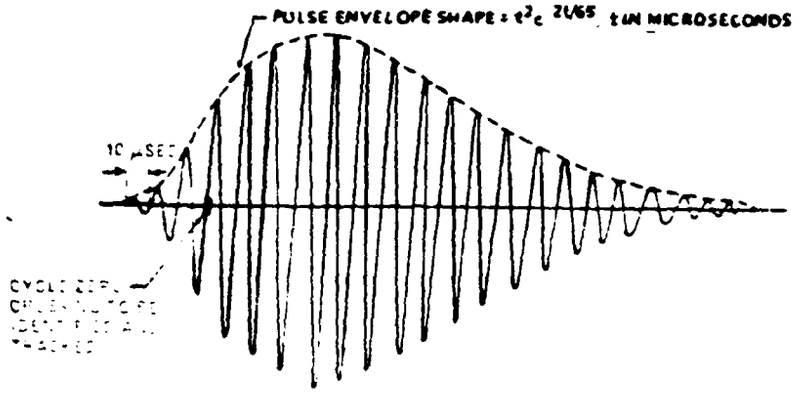
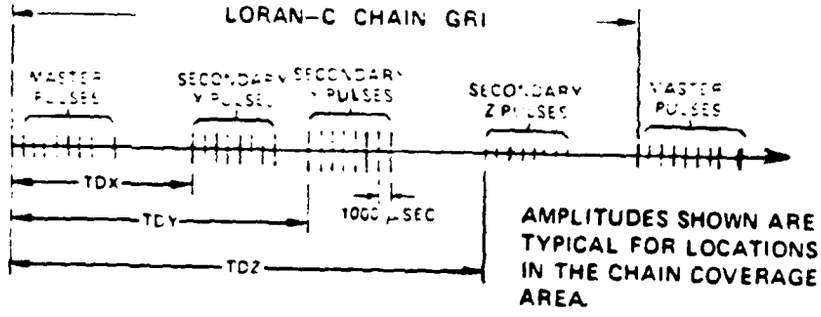


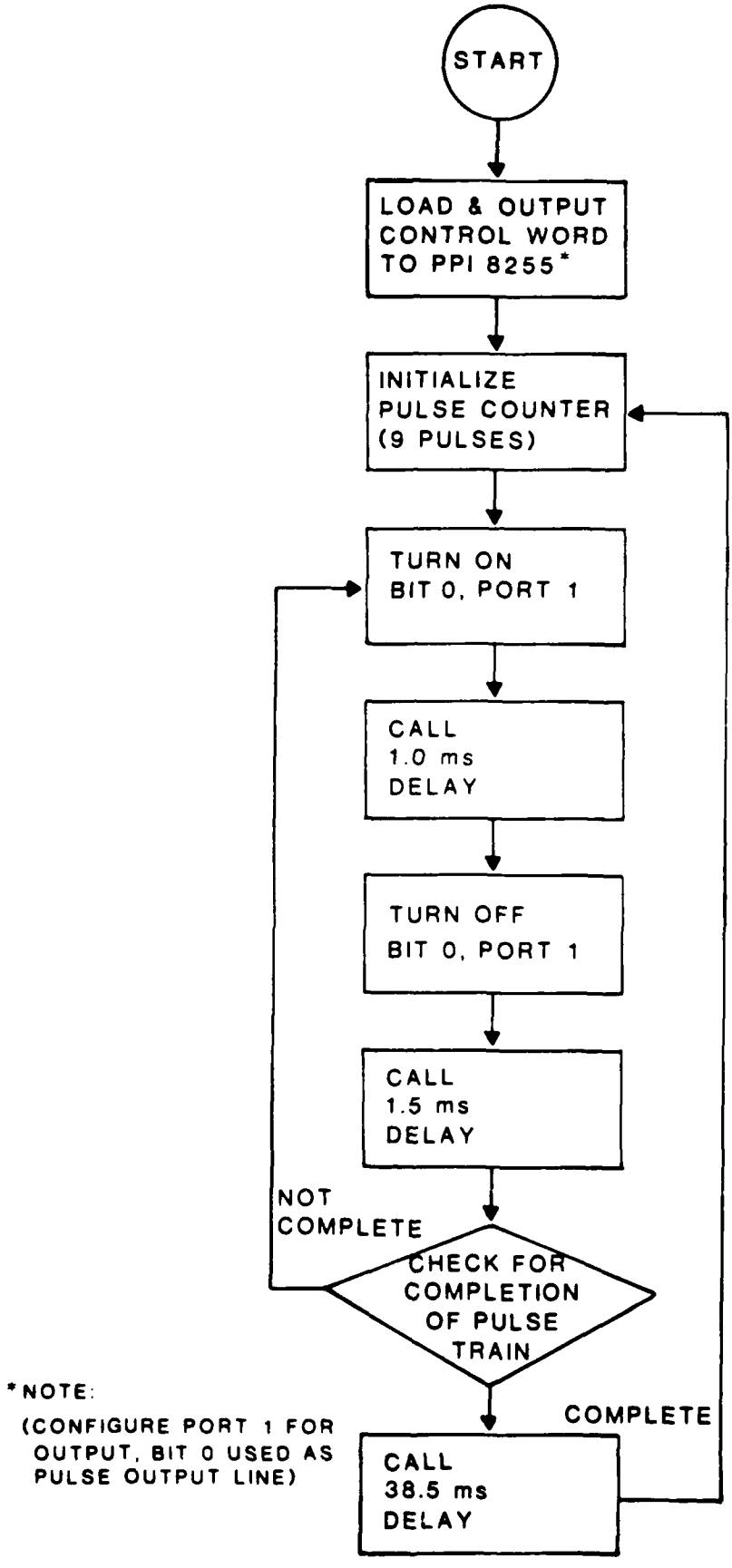
LORAN-C PULSE



EXAMPLE OF RECEIVED LORAN-C SIGNAL

FIGURE 1

Pulse Generator Program



*NOTE:
(CONFIGURE PORT 1 FOR OUTPUT, BIT 0 USED AS PULSE OUTPUT LINE)

FIGURE 2

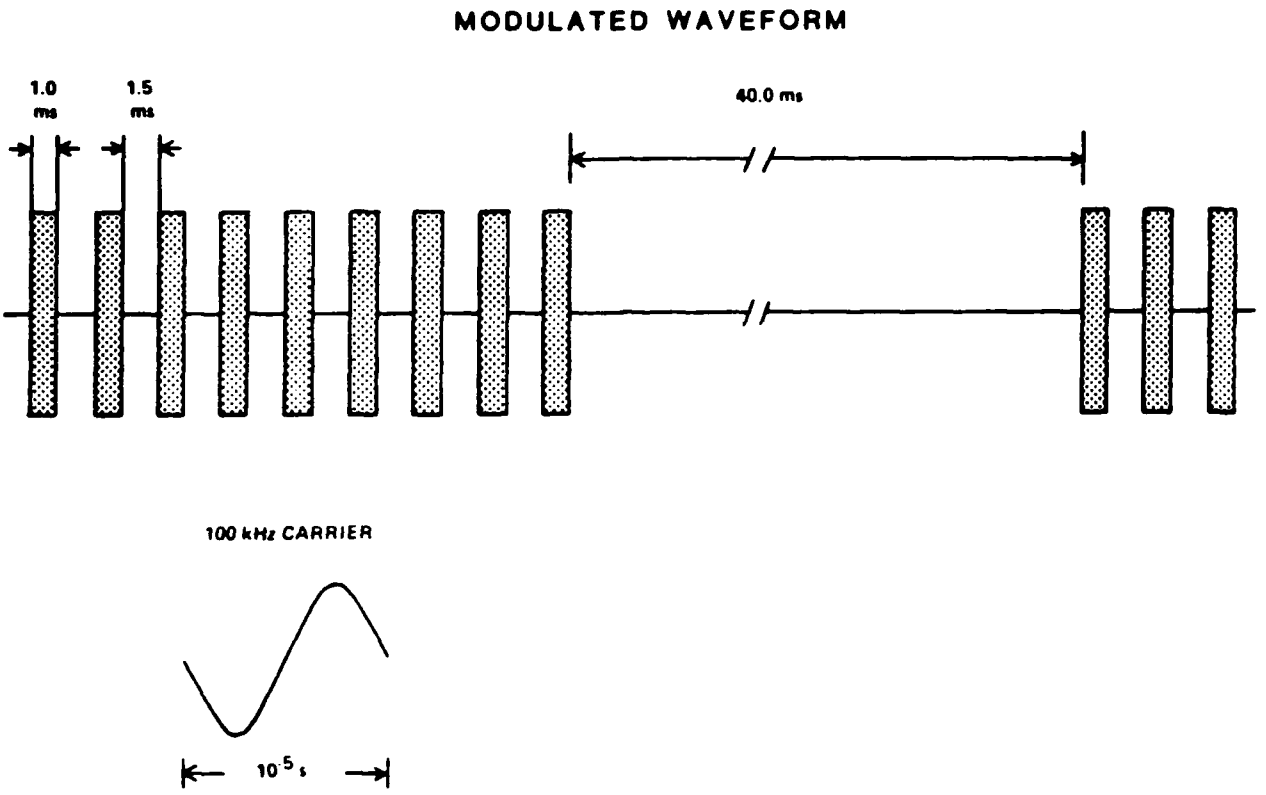


FIGURE 3

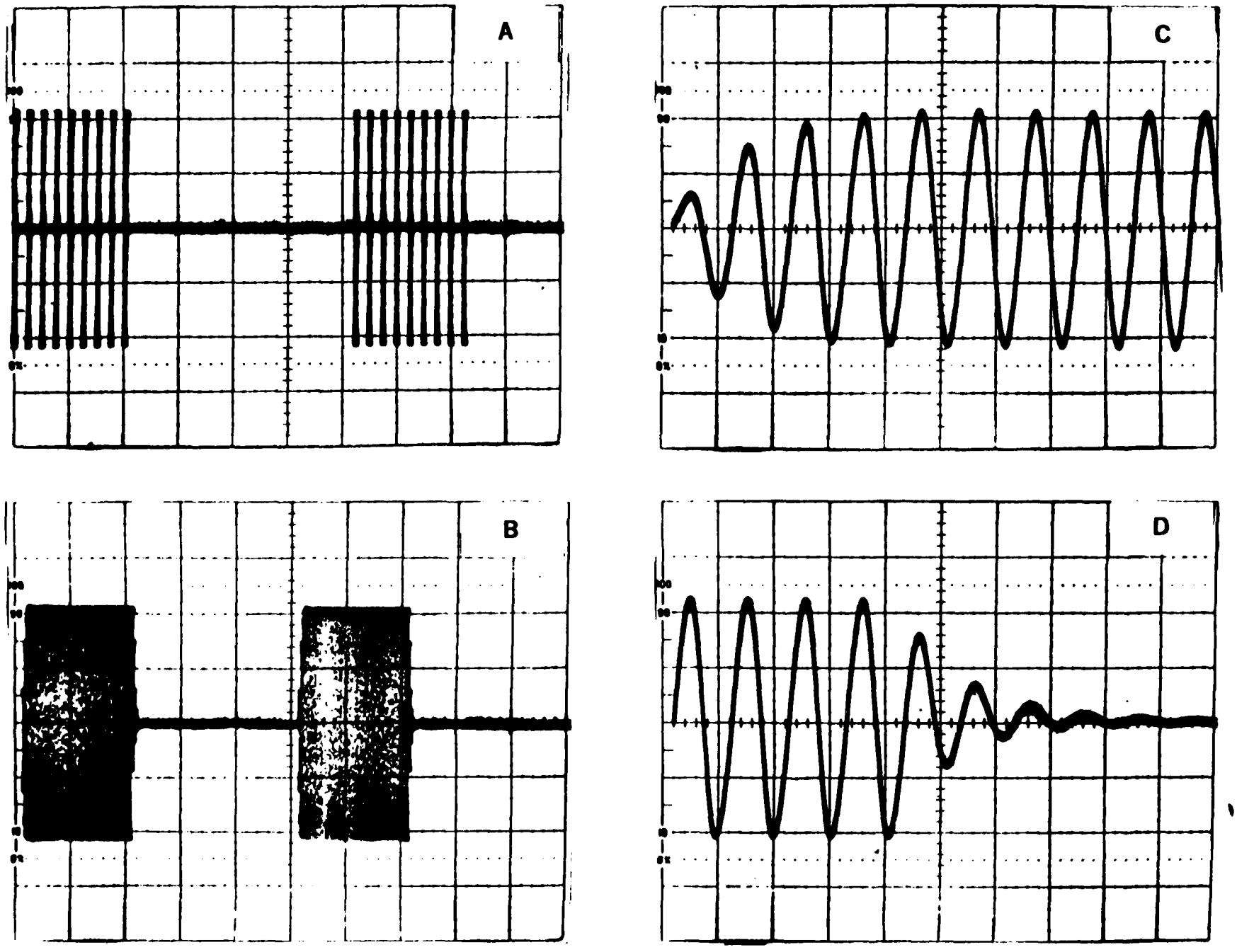


FIGURE 4. Output of the electric field generation equipment

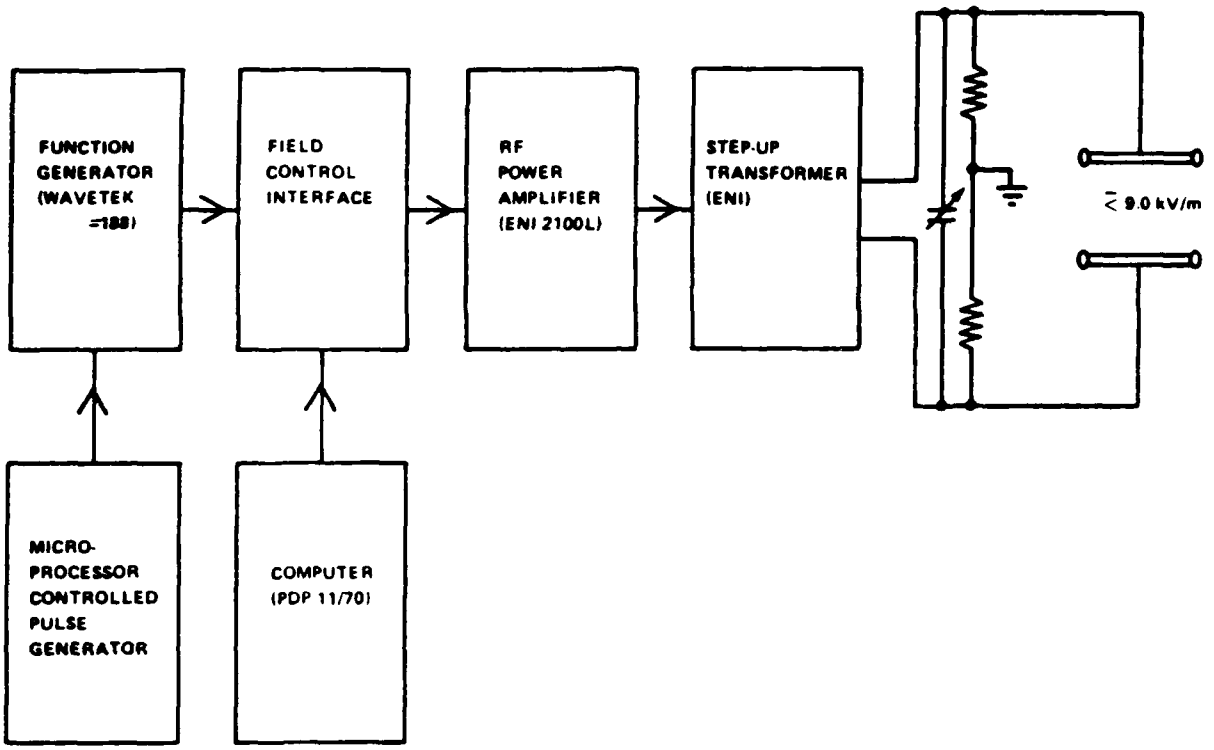
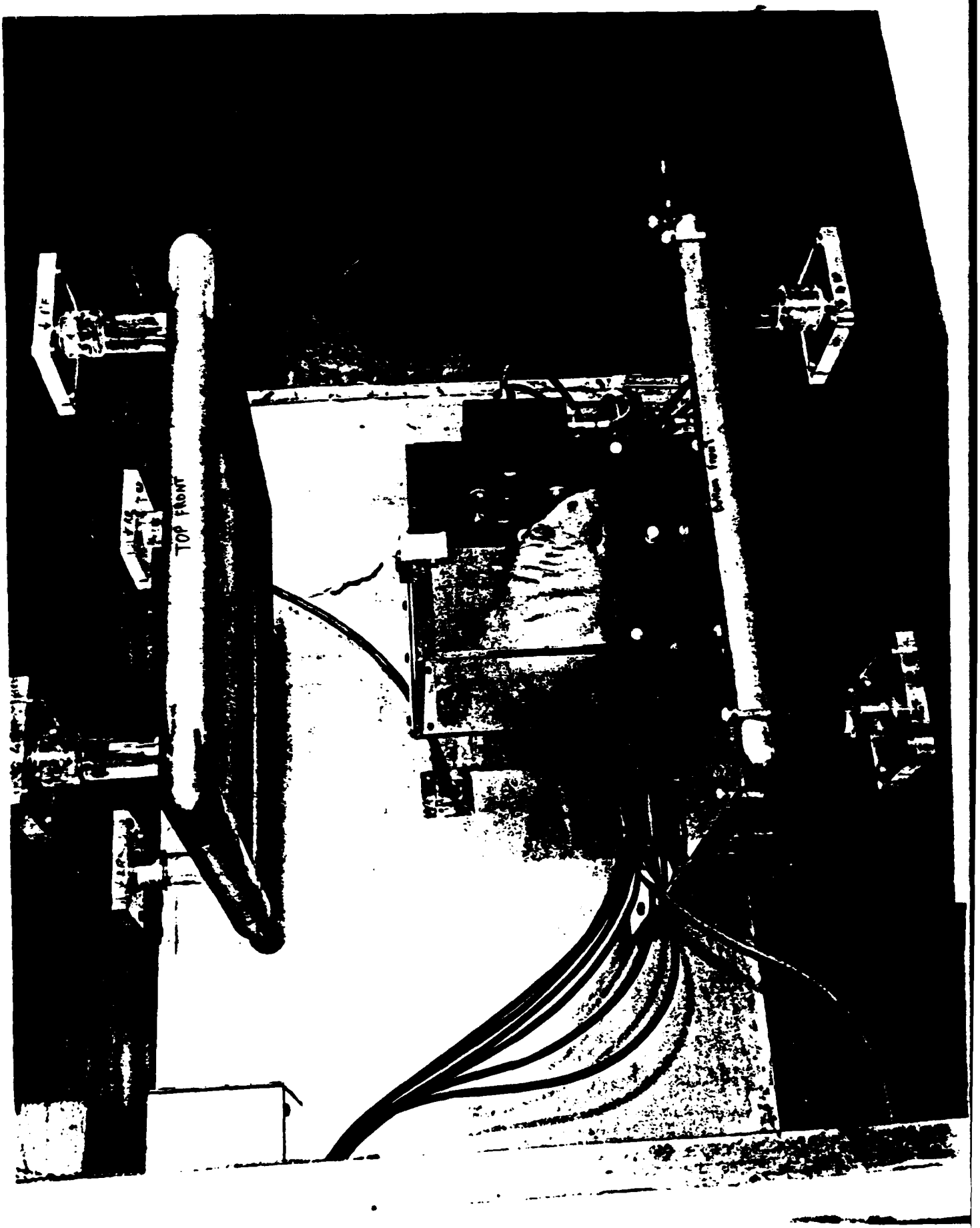


FIGURE 5. Exposure system for the behavioral studies.


TOP FRONT

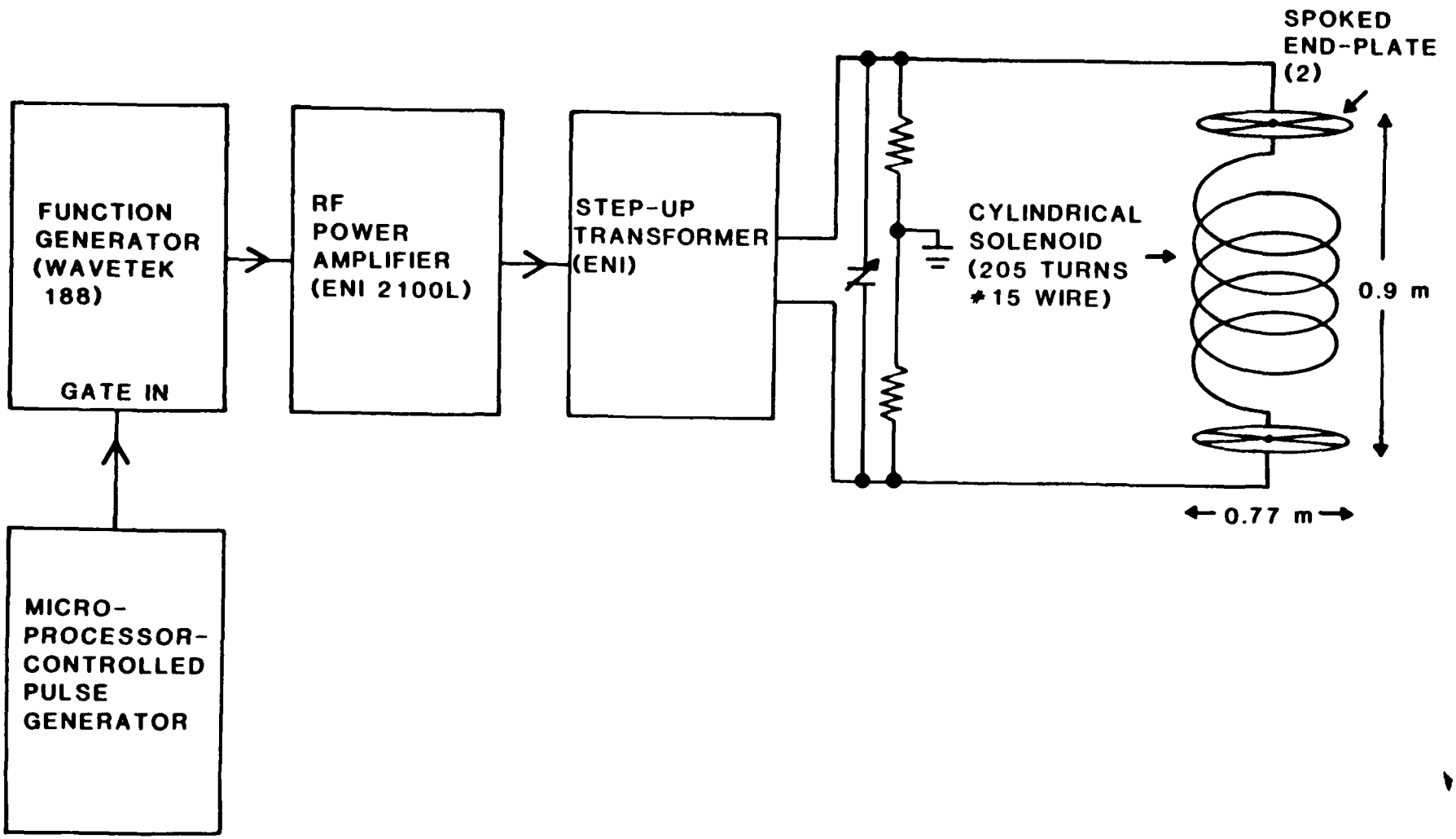


FIGURE 7. Exposure system for the neurochemical studies

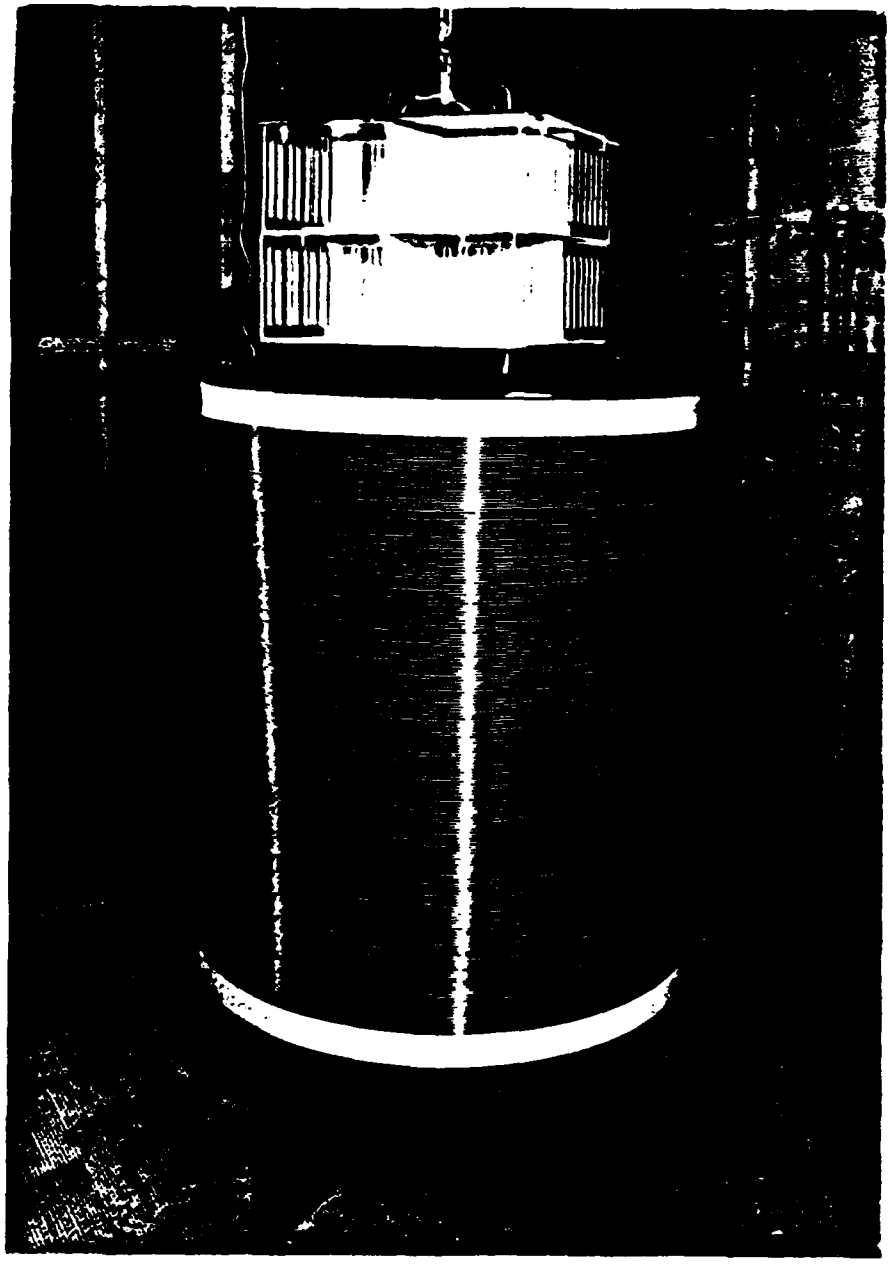


FIGURE 8. Exposure apparatus for the neurochemical studies

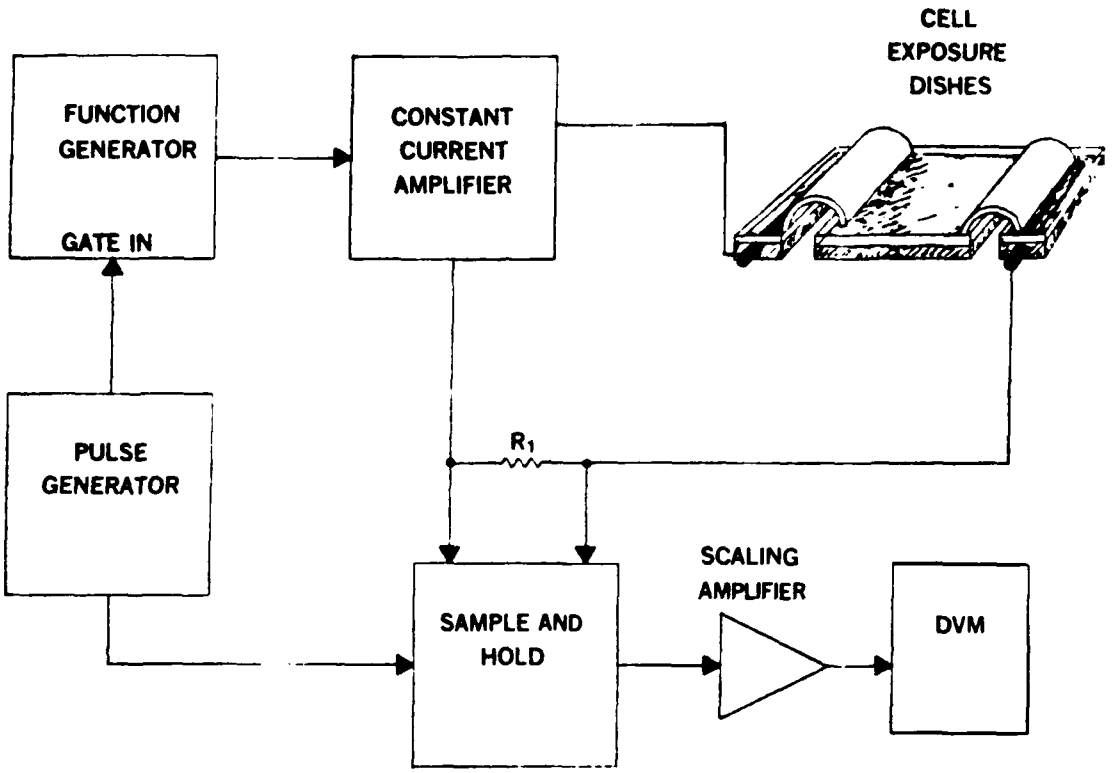


FIGURE 9. Exposure system for cultured tissue

3.0 BEHAVIORAL CHANGES ASSOCIATED WITH EXPOSURE TO kHz FIELDS.

M. E. Stell and P.M. Sagan

3.1 INTRODUCTION

The goal of this research is to determine the behavioral potency of kHz fields in a simple test which has been shown to work with rats exposed to 60 Hz (Sagan et al., 1981). The long-term goal is to proceed beyond a phenomenological account of electromagnetic field effects towards an understanding of the mechanisms by which these effects occur.

3.2 RATIONALE

Of the many behavioral processes available perception seemed the best suited. It is one of the simplest in which the central nervous system makes a significant contribution. Also, and equally important, a well developed methodology for studying perception was already in existence (psychophysics). Having proposed to study the perception of electric fields a number of potential outcomes were examined in order to direct the actual course of the experiment. A study like this could reveal:

1) No effects. The electric fields might not produce behavioral effects. It is important to note that such results would not preclude other biologically relevant effects of electric fields, effects which could be seen at the cell or tissue level, but not at the behavioral level.

2) Non-specific effects. The electric fields might produce global, non-specific changes, such as changes in arousal level. One such effect might be an increase in all motor activities. Such effects would be seen as behavioral changes during the application of the field, but not necessarily as detection of the field.

3) Modified hedonic values. The electric fields might alter the reinforcing properties of food, water, drugs, etc. Such effects might modify the subjects' willingness to proceed with the test without the subject being able to detect the field.

4) Sensory stimulation. The electric field might stimulate the animals' normal sensory systems. Electric fields might be sensed through movement of bodily hair, or, as in the case of strong magnetic fields, produce direct stimulation of the visual system, i.e., magnetophosphenes (Lovsund, et al, 1980). If this were to occur the subjects would confuse the electric fields with other forms of sensory stimulation.

5) Direct sensation of the field. The electric field could be sensed as a unique sensory event. In this case the field would not be confused with other forms of sensory stimuli, nor would there be gross changes in motor activity, nor changes in the reinforcer's hedonic value.

An experiment was designed to separate these potential outcomes. The protocol was to test for the last type of mechanism, direct sensation of the field, but with the potential of identifying the other mechanisms.The methodology was that of classical psychophysics. The specific goals of the experiment were to:

1) Determine if rats can sense kHz electric fields. The first goal was to search for all the mechanisms listed above. If detection of the field occurred then it was planned to proceed with the following goals.

2) Determine if there is a lower threshold (Reiz Limen, RL) for electric fields detection, and if there is a dynamic range over which subjects detected the fields. A dynamic range is present if increased stimulus energy, above RL, improves detection, up to some maximum level (ceiling).

3) Determine the mechanism(s) behind this detection.

3.3 PROGRESS REPORT

METHODS

1. Subjects

The subjects are male Sprague-Dawley rats from B & K or Charles River breeders. They are water deprived 23 hours before testing begins.

2. Trials procedure

The trials procedure is diagrammed in figure 1. Each subjects is tested 5 days a week in sessions lasting one hour per day. During a session the subject repeatedly initiates "trials" which last a minute or two. The subject's task is to indicate whether the stimulus is or is not present during each individual trial. The subject completes the trials by pressing three levers available in the exposure apparatus. Trials are begun by pressing the center lever three times. Then the computer randomly choses to produce either a stimulus trial or a no stimulus trial. The subjects are taught that one of the outer levers is correct if the stimulus is present and the other is correct if it is not. Thus, the subject indicates whether or not he detects the stimulus by which lever is pressed. Initially a tone is used to train the subjects. Later the stimulus is the electric field.

One potential problem with this approach is that the subject can circumvent the procedure. Simple alternation between the two outer levers would eventually be reinforced. To avoid this the subject is penalized for changing levers by a six second "change-over-delay" (COD). The COD makes simple alternation less rewarding. In order to increase the amount of behavior obtained on each trial the subject has to keep pressing the correct lever for a predetermined length of time. The length of time varies in a pseudo-random fashion, on a "variable interval" (VI) schedule. This assures steady, but slow rates of responding and the collection of substantial

amounts of behavior. As an aid to training the subject receives a second reinforcement during each trial if he keeps pressing on the correct lever. However, these presses (after the first reinforcement) are not analyzed. Following the second reinforcement the software waits for the subject to initiate another trial.

The measure of performance used is the "Discrimination Ratio". It is simply the number of correct presses divided by correct and incorrect (i.e, total) presses. This ratio can range from zero (no correct presses) to 1.0 (all presses are correct presses). Chance performance would be 0.5 (equal presses on both levers). Only data from stimulus trials are analyzed. The purpose of no-stimulus trials is to force the subjects to decide if a stimulus is actually present or not.

3. Apparatus

The equipment used in these experiments is described in detail in the Section 2.0 (EMF Parameters and Instrumentation) of this document.

RESULTS

The results of the behavioral experiments to date are shown in figures 2a-b and 3a-b. At the time of the final report (Dec. 1985) a pilot study had been completed in which two subjects had be fully trained and tested. The training and testing for these two subjects, R85010 and R85015, occurred on the following schedule:

1) Dates: 18-MAR-85 through 1-APR-85, Condition: Initial training in the test chamber. Schedule: CRF.

2) Dates: 2-APR-85 through 22-APR-85, Condition: Introduction of a short variable interval (VI) schedule. The full range of the tone is divided into 20 different levels, with one of the levels being randomly selected for each stimulus trial. Schedule: VI2, Tone Only as the training stimulus.

3) Dates: 23-APR-85 through 26-APR-85, Condition: Lengthening VI. Schedule: VI6, Tone Only as the training stimulus.

4) Dates: 27-APR-85 through 4-AUG-85, Condition: Delaying testing while final equipment fabrication and software development takes place. Schedule: None.

5) Dates: 6-AUG-85 through 16-Aug-85, Condition: Continuing testing on the VI6 schedule, but now tone and electric field co-vary. The full range of electric fields, like tone stimuli, is divided into 20 different levels. On each stimulus trial one level of the tone is presented and the corresponding level of the electric field is also presented. Thus, the rat is allowed to respond on the stimulus correct lever in the presence of the tone and electric field. Schedule: VI6, Tone and Field as the training stimuli.

6) Dates: 19-AUG-85 through 23-AUG-85, Condition: Lengthening VI. Schedule: VI8, Tone and Field as the training stimuli.
7) Dates: 26-AUG-85 through 28-AUG-85, Condition: Lengthening VI. Schedule: VI10, Tone and Field as the training stimuli.

8) Dates: 29-AUG-85 through 5-SEP-85, Condition: Tone being faded out while leaving the field at full strength. Schedule: VI10, Tone reduced 5 dB-SPL from the maximum possible, Field full strength.

9) Dates: 6-SEP-85 through 24-SEP-85, Condition: Tone further reduced while leaving the field at full strength. Schedule: VI10, Tone reduced 10 dB-SPL from the maximum possible, Field full strength.

10) Dates: 25-SEP-85 through 24-OCT-85, Condition: Field only. Actual testing occurs during this time period. Schedule: VI10, Field Only as the testing stimulus.

The results are presented at two stages in the experiment. The data from the tone only phase are presented in figure 2a-b. This procedure worked reasonably well with the tone as the stimulus. The average slope of the line is positive, meaning that as the stimuli became more intense the subject pressed more often on the stimulus correct lever and less often on the no-stimulus correct lever. Random fluctuations in their performance can be seen in the data, but this is due to a relatively small sample size. Thus, both subjects showed a very normal type of psychometric function for this stimulus (sound) and for this species (rat).

Figure 3a-b represents the data from the field-only phase of testing. In this figure the apparent slope of the line is not positive, but rather zero. And, the overall index of performance, the discrimination ratio stays remarkably close to chance performance (a ratio of 0.5). In the case of subject R85010 the ratio is actually slightly below 0.5. In the case of R85015 the ratio fluctuates around 0.5. The lack of slope in the psychometric functions of these two animals suggests that increasing the intensity of the Loran C type electric field does not improve performance. The fact that both animals' performance remained remarkably close to chance at all field levels is strong evidence that these subjects were not detecting the field.

In summary, the data from this pilot study do not indicate that the Loran C type electric field was detectable by the subjects. However, these data represent only two subjects. There are numerous possible explanations for the difficulty these subjects experienced when trying to detect the field. One explanation is that the field is not detectable. Another explanation is that the particular experimental parameters were not suitable to demonstrate detectability.

3.4 PROPOSED RESEARCH

As outlined above, the current research on the behavioral aspects of the Loran C type electric field has indicated that this field was not detectable by our subjects at the conditions used. An examination of the experimental protocol might be a reasonable next step. There are several parameters of interest that could affect the eventual outcome of these experiments.

3.4.1 Insufficient Field Strength

It may be that the strength of the field being used is not sufficient to reveal its detectability. There are two reasons not to immediately pursue this line of investigation:

1) The maximum voltage levels are within the level found to be detectable for sine wave 60 Hz electric fields using similar procedures with similar subjects. Also, the maximum current through a capacitor plate system such as this one is frequency dependent. The current at 100 kHz is expected to be about 3 orders of magnitude larger than the current at 60 Hz. Thus, both the voltage and the current levels would seem to be equivalent to or greater than those known to be detectable.

2) The voltage levels used in this experiment are substantially higher than those reported during a site survey of Loran C broadcasting sites (McEnroe, 1980). McEnroe reported a maximum electric field to be produced only very near the antenna feed line and the the root sum of squares of the voltage was about 2.7 kV/m. Our study is uses electric fields as high as 25 kV/m (p-p) for the unmodulated portion of the 100 kHz carrier.

3.4.2 Insufficient Duty Cycle

It may be that, even if the voltage and current are of sufficient intensity to be detectable, the relatively short duty cycle could make the Loran C type waveform used in these experiments hard to detect. However, the duty cycle is similar to the actual Loran C duty cycle. Therefore, alteration of this parameter would lead to some difficulty in interpreting experimental results relative to an actual Loran C exposure. It would seem prudent, therefore, that alterations to the waveform or waveshape be reserved until other forms of possible procedural problems are examined.

3.4.3 Insufficient Behavioral Protocol

It is also possible that the detection of the Loran C type waveform is possible for these subjects, but that procedural problems are masking this detection. For example, it may be possible that detection of the Loran C type waveform is simply more difficult than detection of a comparable amplitude 60 Hz sine wave field. In that case the subjects should be trained with the utmost care at each step of the procedure, and an experimental procedure should be used that maximizes the possibility that the subjects will not be exposed to large numbers of sub-threshold trials; trials in which the subjects are rewarded for indicating that the field is present even when they cannot in fact determine that it is.

In the pilot study the experimental protocol was to select the amplitude of the stimulus in a pseudo-random way, regardless of the subject's performance. This technique is called the method of constant stimuli and is a well established technique in psychophysics. However, if the range from which the stimuli are chosen contains many levels below threshold, the animal will often be required to press the stimulus correct lever on sub-threshold trials even though he does not detect the field. These trials will serve to destabilize the animal's behavior. Another method that is commonly used in psychophysics, and one that is appropriate if the range of stimuli contain many sub-threshold values, is the "tracking" method used by von Bekesy, 1947. In this method each session begins with a large amplitude stimulus that is easily distinguished from the no stimulus condition. The stimulus is then reduced by a fixed increment over a number of trials until it is not reliably distinguished from the no stimulus condition. Then the stimulus is increased by a relatively large amount. This down-up-down cycle is repeated over and over throughout the test session. This method has the advantage of reacting to the performance of the animal as the experiment is in progress.

CONCLUSIONS

In summary the current subjects have not given any indication of the ability to detect the Loran C type field. This could be due to a number of reasons, some of which are discussed above. It would seem logical that the experiment be replicated, but using a slightly different experimental protocol and taking care at each stage of the experiment to make sure that the subjects are ready to proceed to the next stage. The proposed protocol, the tracking method, when properly and judiciously applied, is one of the most sensitive indicators of detectability of sensory stimuli. If Loran C type waveforms are actually detectable by rats at these field amplitudes then it would be expected that this method would maximize the likelihood of finding such detection.

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Figure Captions

Figure 1. The course of a single trial is diagrammed (see figure for caption).

Figure 2a-b. Psychometric functions for subject R85010 and subject R85015 while detecting the tone stimuli. On the X-axis is tone intensity, ranging from 68.43 decibels-Sound Pressure Level (dB-SPL) to 90.00 dB-SPL. On the Y-axis is the subject's performance, represented as the ratio of correct presses divided by total (correct and incorrect) presses. This figure summarizes 17 days of testing with tone. A line is drawn through the figures at the level of chance performance (0.5 discrimination ratio). These subjects appears to begin detecting the tone at about 70-75 dB-SPL. For tones louder than this performance increases at the loudness increases.

Figure 3a-b. Psychometric functions for subject R85010 and subject R85015 while detecting the Loran C type waveform. On the X-axis is the strength of the Loran C type electric field. Field strength here is measured as the peak-to-peak voltage seen on the exposure plates during the unmodulated portion of the waveform (about 3 kV), divided by the distance between the plates (.33 m). On the Y-axis is the usual measure of the subjects' performance. This figure sumarizes 19 days of testing with the field. These subjects show no performance above chance levels, and no tendency for performance to improve as the strength of the field is increased.

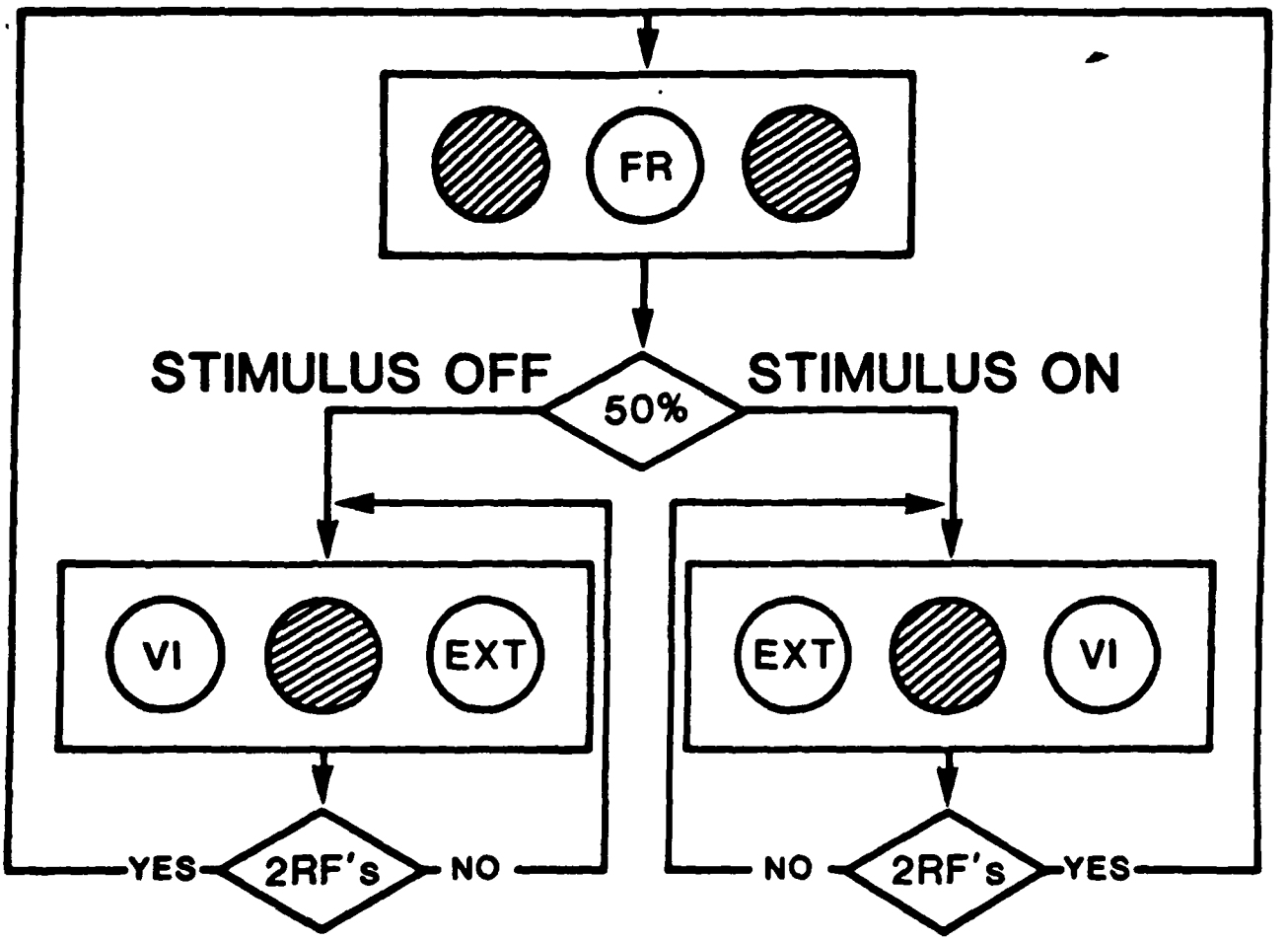

Figure 1. The course of a single trial is diagrammed. At the top of the figure the three press panels are depicted as they would occur between trials. The center press panel is illuminated while the outer two are dark. When the subject presses the center panel three times, on a Fixed Ratio 3 schedule, ("FR") the computer will start a trial. The computer then randomly decides to present either a no-stimulus trial ("stimulus off"), or a stimulus trial ("stimulus on"). In either case the center panel is darkened and the outer two panels are illuminated. The subject is taught to press one lever if the stimulus is present and to press the other lever if the stimulus is not present. For example, if the computer selected a stimulus trial the conditions in the lower right would apply. The subject would be rewarded for pressing the right hand panel on a Variable Interval schedule ("VI"). He would never be rewarded for pressing the left hand panel, i.e. this behavior is extinguished ("EXT"). During a stimulus off trial the left panel is correct ("VI") while the right hand lever is incorrect ("EXT"), see bottom left. The important concept is that the subject is taught to press one lever if the stimulus is present and to press the other if the stimulus is not present.

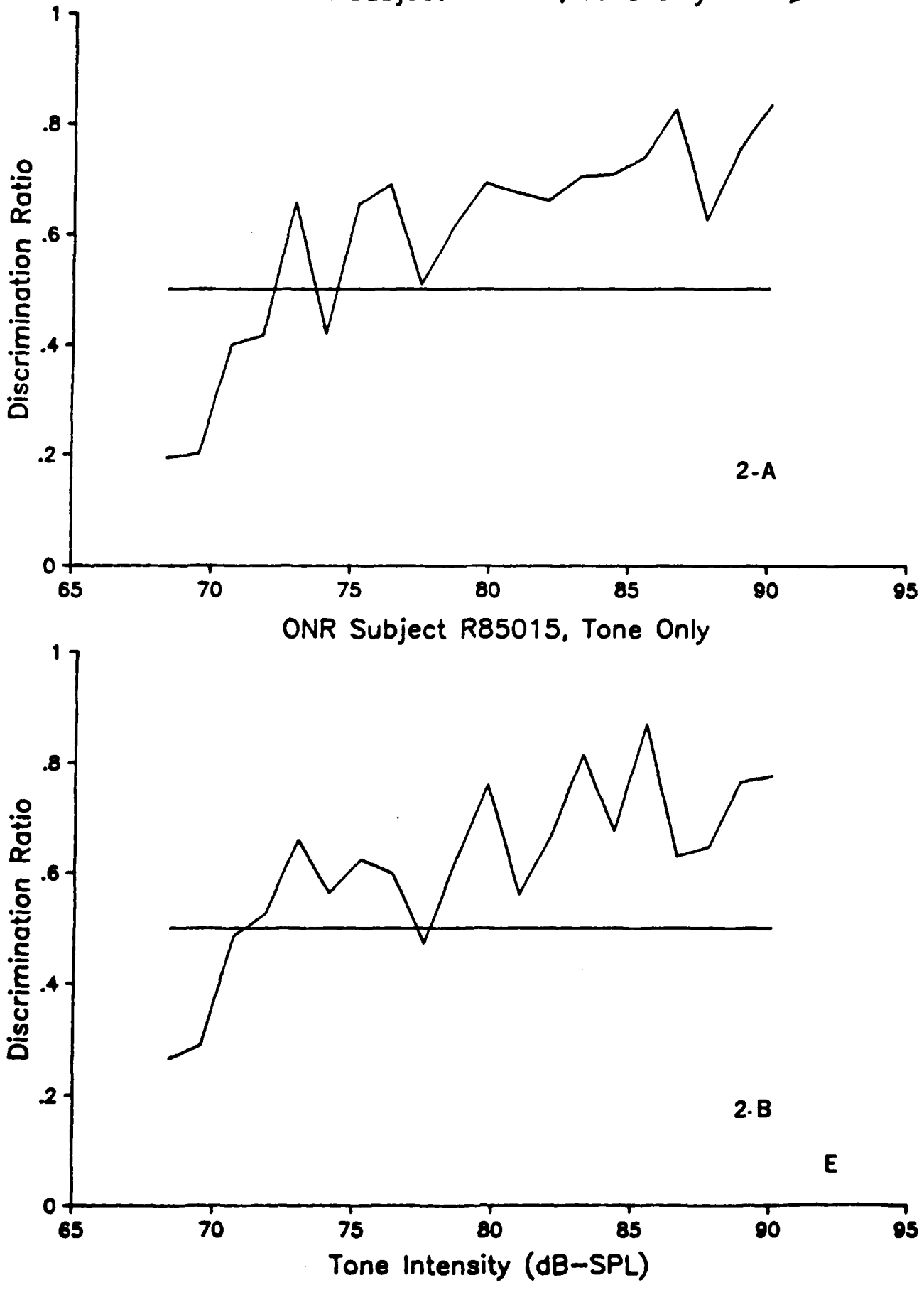
Psychometric Function
ONR Subject R85010, Tone Only
Discrimination Ratio
1
.8
.6
.4
.2
0
65
70
75
80
85
90
95
2-A
ONR Subject R85015, Tone Only
Discrimination Ratio
1
.8
.6
.4
.2
0
65
70
75
80
85
90
95
2-B
E
Tone Intensity (dB-SPL)

FIGURE 2

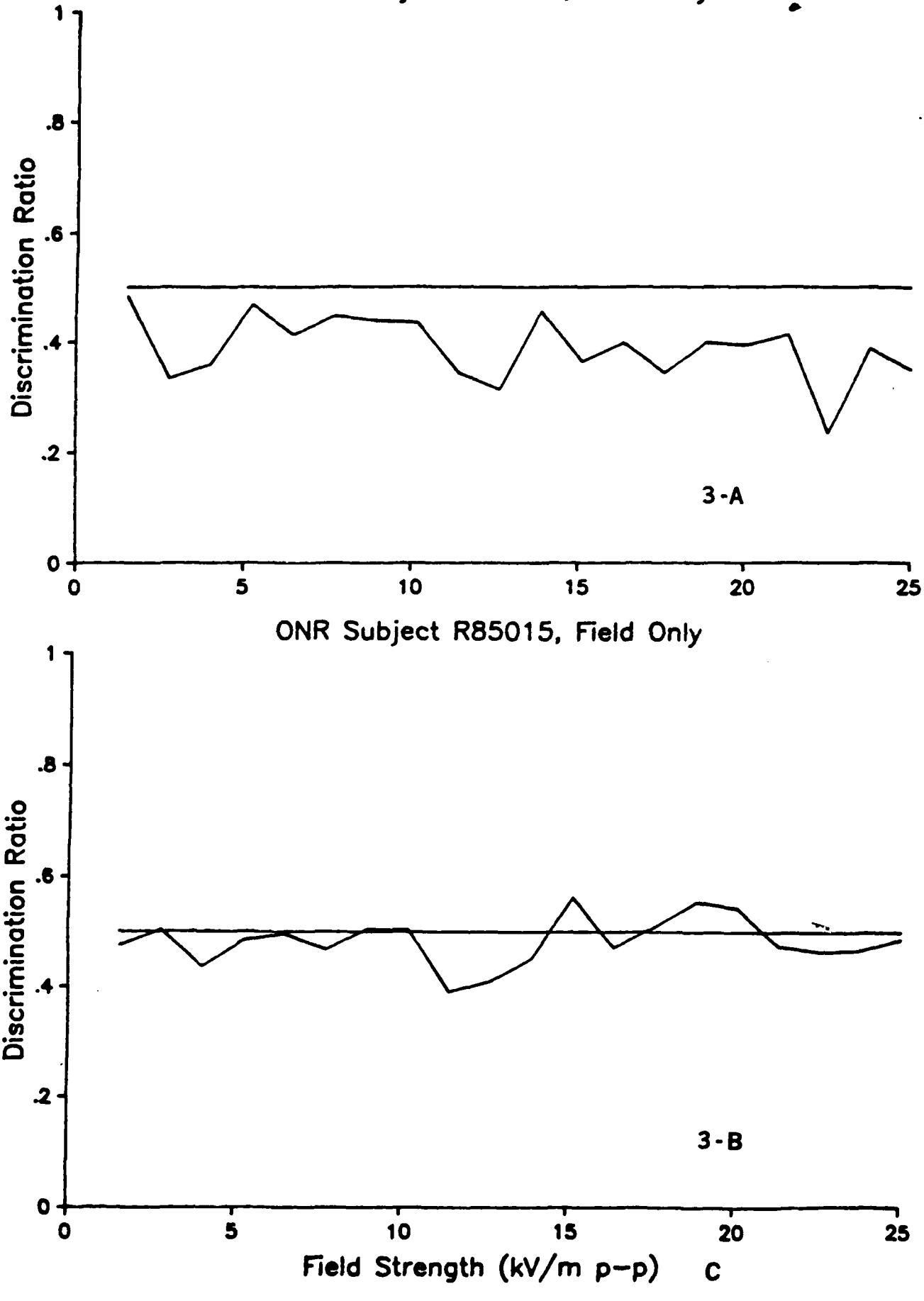
Psychometric Function
ONR Subject R85010, Field Only
Discrimination Ratio
1
.8
.6
.4
.2
0
0
5
10
15
20
25
3-A
ONR Subject R85015, Field Only
Discrimination Ratio
1
.8
.6
.4
.2
0
0
5
10
15
20
25
3-B
Field Strength (kV/m p-p)
C

FIGURE 3

4.0 NEUROCHEMICAL CORRELATES OF kHz EMF EXPOSURE.

B.J. Vasquez, and S.M. Bawin.

4.1 INTRODUCTION

The goal of this research is to monitor brain neurotransmitter substances after single and repeated in vivo exposure to combined electromagnetic fields. The biogenic amine systems that we studied in the rat brain are correlated to important mental, vascular, and neuroendocrine functions in man.

4.2 RATIONALE

Although there is a body of evidence showing that exposure to electric and magnetic fields can alter the synaptic function and by consequence the nervous system integrity, the neurohormonal correlates of those changes have not yet been systematically studied.

The availability of neurotransmitter substances as well as the number and sensitivity of their pre and postsynaptic receptors are of main importance in the sequence of neural transmission. To address the question of effects of in vivo exposure to EMFs we proposed to study the biogenic amine and opiate receptor systems in rat brain tissues.

4.3 PROGRESS REPORT

In compliance with the revised pert chart for proposed research sent to Dr M. Marron (August 17, 1983) we have developed all the chemical techniques required and they are routinely used in our laboratory. The development and construction of equipment has taken most of the contracting period and it is detailed in the Section 2.0 (EMF Field Parameters and Instrumentation) of this document. We are now reporting the completion of the exposure systems for the neurochemical series of studies to start after the arrival of animals purchased in January, 1986. We plan to have collected data to report on the contract anniversary date in April.

In the past years we have used the developed chemical procedures in projects supported by the Bureau of Radiological Health, the Department of Energy, and Southern California Edison. We are including here the reports of that research as a background to the work to be completed for ONR.

4.3.1 LEVELS OF BRAIN BIOGENIC AMINES FOLLOWING IN VIVO E-FIELD EXPOSURE

To define neurochemical correlates of EMF effects we monitored brain regional biogenic amines in male albino rats continuously exposed to 60 Hz electric fields of 10 to 12 kV/m (RMS) magnitude in air. Animals were subjected to this treatment for different lengths of time: 1-day, 1-week, or 4-week.

METHODS

1. Exposure apparatus

Vertical 60 Hz electric fields were induced between two parallel metal plates (2m x 1m) covered by 1/8 inch Plexiglas sheet and separated by a distance of 0.5m (Fig. 1). Ten animals were singly housed in two rows of five plastic cages located on the lower plate. Fields inside the cages, measured with a miniature probe designed by Misakian et al. (1978), ranged from 11 to 12 kV/m. Corona was not present visually nor as noise in the AM radio band. An identical apparatus was used for the sham condition with a zero applied field. Room temperature (22°C) and relative humidity (60%) were recorded throughout the experiment.

2. Animals

Male albino rats (Sprague-Dawley) from Simonsen (Gilroy, CA) were used for this study. They received ad lib food and deionized water and were provided a 12:12 hour light cycle (lights on at 6 AM). They were changed to clean cages every 48 hours.

3. Detection of biogenic amines

Animals were sacrificed by decapitation at the end of exposure. Brains were rapidly dissected into several regions (striatum, hypothalamus, hippocampus and frontal cortex) by the method of Messing et al. (1980). Tissues were kept at -70°C until assayed by HPLC-ECD (high performance liquid chromatography with electrochemical detection) methods.

Norepinephrine (NE), epinephrine (E), dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), together with serotonin (5HT) and its metabolite 5-hydroxyindolacetic acid (HIAA), were assayed in each sample. Adrenal glands were also assayed to test for possible generalized stress effects.

Tissues were homogenized for 20 sec in perchloric acid with a sonicator probe at low power while kept ice cold. Centrifugation and microfiltration of the samples preceded their injection onto the HPLC system (Plotsky et al., 1977).

RESULTS

A significant percentage of research effort was spent in mapping brain regional biogenic amines in animals exposed to 60 Hz electric fields. In a series of pilot studies, sham and experimental groups of rats were subjected to different length of continuous exposure (1 day, 1 week or 4 weeks) to a 14 kV/m electric field. A grounded cage control group was also used. The group size for this study was determined by the availability of exposure chambers that were shared with the behavioral laboratory under the supervision of Dr. P.Sagan. Two cage control, two sham and four experimental (field on) animals were included in each of the exposure length groups.

Since cage control and sham animals showed no differences they were combined for statistical purposes making n=4 for both the control and experimental groups. Student's t-tests of the data did not show any statistically significant difference in tissue concentrations of biogenic amines between groups at any of the conditions under study. A possible explanation for these results might be that the population tested was too small to unmask subtle effects. An alternative explanation is based on the fine homeostatic mechanisms of the brain that enable the system to keep levels constant by balancing changes in the rate of use with changes in the rate of synthesis.

The next study included higher number of animals (10 per experimental condition) exposed in the system described in the Methods section to a 60 Hz electric field of 14 kV/m magnitude in air, for either 1 day or 1 week, and its corresponding shams similarly housed. Levels of NE were found significantly decreased in striatum and hippocampus after 1 week but not after 1 day exposure. These effects appeared independent of field-induced stress, since the adrenal concentration of catecholamines was unchanged. Our preliminary results agreed with data obtained by the Battelle group using 65 kV/m exposures for one month and they were reported at the 1984 Soc. for Neuroscience meeting (Hilton et al, 1984; Vasquez et al, 1984b). After repeating the experiment on animals exposed to the same field for 1 day, 1 week and 4 weeks, neither we nor Hilton et al. could replicate the changes in NE previously observed. Tests of the data did not show any statistically significant field-related difference in tissue concentrations of biogenic amines in any of the conditions under study.

Metabolites are an index of physiological activity. Levels of metabolites of the DA and 5HT systems were expected to reflect any field effect that might alter the rates of utilization and synthesis that are balanced by homeostatic mechanisms of the brain. However, our results did not indicate any significant difference between exposed and sham animals.

4.3.2 DAILY RHYTHMS OF NEUROTRANSMITTERS FOLLOWING IN VIVO E-FIELD EXPOSURE

Concentrations of neurotransmitters as well as concentrations of their synthetic enzymes and metabolites, vary over the course of a day. In the rat brain there are also circadian and seasonal rhythms in the number of neurotransmitter receptors (Kafka et al., 1983)

METHODS

1. Exposure system

To detect and quantify the possible effects of 60 Hz electric fields on circadian rhythms we collaborated with researchers at Battelle Pacific Northwest Labs., Richland, WA.

The equipment used has been described in detail by Free et al.(1981). Briefly, rats were exposed to uniform, vertical, 60 Hz electric fields in a parallel-plate rack system. Each rack contained four horizontal, planar,

1.0 m x 2.2 m electrodes stacked vertically with a spacing between electrodes of 40 cm.
Rats were housed singly in 12 cm wide x 25 cm long x 10 cm high polycarbonate cage modules with stainless steel screen floors mounted on the electrodes on which they were standing. Field strength was continuously recorded. The nominal 60 kV/m E-field resulted in a 39 kV/m effective dose to each animal. Sham animals were housed in a similar but inactivated system. Room lights were on a 12:12 cycle (lights on at 7 AM). Temperature (22°C) and relative humidity (40%) were also continuously recorded.

2. Animals

Sprague-Dawley (Charles River) male albino rats were exposed 20 hours a day for four weeks. On the sampling date, animals were sacrificed in groups of 6 Sham and 6 Exposed every four hours throughout the day starting at 6 AM. Six brain areas from each animal and four pair of adrenal glands at each time point were dissected, stored in cryogenic vials, and immediately submerged into liquid nitrogen. All tissues were packed in dry ice and transported to Loma Linda for chemical determinations.

3. Detection of biogenic amines

The levels of biogenic amines and their acid metabolites in striatum, hypothalamus, hippocampus, and adrenal glands were determined by HPLC-ECD methods. The brain stem, anterior and posterior cortices were used for assays of opiate receptor binding. This was a double-blind experiment with a code (kept at Battelle) to be broken only after all the tissues have been processed. Partial information was needed to organize the results and we now have data divided into two groups, RED and BLUE, one of which is the treated group, but the code is still unknown to us in Loma Linda.

4. Opiate receptor binding

Binding assays were performed according to a modification of the method of Pert et al. (1973). Membrane preparations were obtained by homogenizing brain tissue areas in ice-cold Tris buffer (pH 7.4, 0.05 M), followed by washes and centrifugation. Aliquots of resuspended membranes in a 1:10 (tissue:buffer) solution were incubated for 45 min at room temperature with [^{3}H]naloxone in the presence or absence of an excess of levallorphan. After incubation, samples were rapidly filtered (Whatman GF/B filters) under vacuum (Millipore manifold) and filters were counted in 5 ml of Aquasol. Specific binding was defined as the difference in amount of bound [^{3}H]naloxone with and without levallorphan. For the Scatchard analysis we used 5 or 6 different concentrations of labelled naloxone.

RESULTS

1. Biogenic amines

Two way analysis of variance (ANOVA) tests done on the data expressed as pg/mg wet tissue indicated significant changes over time of day for the levels of amines and metabolites in the striatum and hypothalamus. A RED vs BLUE difference was found for DOPAC in the striatum.

A highly significant interaction between type of treatment (sham or exposed) and time of day occurred for NE in the hypothalamus. This interaction is produced by the crossing over of NE levels at 2 PM (see Fig. 2). In order to more clearly visualize shifts in the amine concentrations during the day, we normalized the data by expressing them as percent change of the 24 h mean levels. To date we have values only from the hypothalamus and have found statistically significant differences between groups for NE and DA at 6 AM, 2 PM, and 10 PM (Fig. 2).

2. Opiate receptors

Preliminary data obtained after continuous exposure of rats for a week to a 14kV/m electric field suggested different field effects on the number of opiate receptors at two different times during the day: 8 AM and 8 PM. Consequently, we ran a more complete circadian rhythm study exposing the animals at the Battelle facilities as described in the Methods section.

Homogenates of brain stem and posterior cortex were used to obtain Scatchard plots with one of six concentrations of [^{3}H]naloxone ranging from 0.125 to 2 nM. Calculations provided the number (Bmax) and apparent affinity (Kd) of the receptors at each time point tested. Five to six independent Scatchard plots (one for each animal) were obtained for each time of day, for each region, and then mean binding constants were calculated. The amount of homogenate from anterior cortex was not sufficient for the kinetic studies so we used only one concentration of the tritiated naloxone (1nM) to assay for specific binding.

To date we have analyzed only data from the brain stem at two times, 6 AM and 10 PM. A significant difference ($p < 0.05$) in Kd values was found between the time of day but not between RED and BLUE groups. A higher affinity of the receptors was present at 10 PM (Fig. 3). However, the number of receptors were unchanged either with time of day or exposure condition.

4.3.3 RELEASE OF BRAIN CATECHOLAMINES FOLLOWING IN VITRO EXPOSURE TO AN ELF-MODULATED RF FIELD

Rat brain slices were incubated in oxygenated physiological buffers. It is well established that these preparations retain the metabolic characteristics of neuronal tissue and are widely used to study in vitro release of neurotransmitters, a Ca^{++} dependent process. Measurement of the amount of neurotransmitter released from a given area of the brain provides an indication of the basal neuronal activity as well as the response of the tissue to different treatments (Becker & Ramirez, 1980).

1. Exposure system

Minced brain tissues (striatum, hypothalamus, and hippocampus) were exposed in a Crawford cell to low level (1.0 mW/cm^2 average power, 5.0 mW/cm pep) 450 MHz fields amplitude modulated at sinusoidal low frequencies (16, 60 and 500 Hz, 85% depth), and unmodulated (CW) (Fig. 4).

2. Detection of catecholamines

The release of NE and DA was measured by HPLC methods. Freshly minced brain tissues were incubated at 37°C for 10 min (during field exposure) in a physiological solution containing normal (5mM) or high (55 mM) KCl. The higher K^+ concentration stimulates the release of neurotransmitter substances by causing cell depolarization and it was used as a check of tissue viability. Catecholamine release from the tissues was then stopped by cold and acid treatment and an aliquot injected onto the HPLC. Only data from samples showing increased release evoked by high K^+ were incorporated in the statistical analysis. All results were analysed by comparison with a sham-exposed group.

RESULTS

We have found conflicting results throughout the series of studies included under this goal but they all suggest that synaptic events can be affected by low level electromagnetic fields, dependent on the modulation frequency.

A first experiment performed at low level (1.0 mW/cm^2 pep) 450 MHz fields unmodulated (CW) and modulated at 16 or 60 Hz showed no statistically significant differences in release of catecholamines between the field treated tubes in the Crawford cell and control tubes in an incubator (Vasquez et al, 1983).

The second experiment was run with the addition of K+ in the incubation solution as described in the Methods section. Two-way ANOVA showed a significant F value for field effect on NE [$F(3,130) = 8.02$, $p<0.001$] and DA [$F(3,146) = 6.40$, $p<0.001$] release in the striatum (Vasquez et al, 1984a). Student's t-tests indicated a decreased release of amines in the ELF modulated fields.

A third experiment was designed to include an additional frequency modulation of 500 Hz to the same RF field. Examination of the release levels of DA from striatum showed a statistically significant field-related change [two-way ANOVA: $F(4,256) = 7.41$, $p<0.001$]. Student's t-tests indicated a significant increase in DA release only from the tissues exposed to the 450 MHz field modulated at 500 Hz (Fig. 5). Although we saw a trend to increased release with increasing frequency of modulation, the examination of levels of NE from the hypothalamus and hippocampus did not show any statistically significant field-related change (ANOVA and t-tests).

However, a replicate study failed to show the increase of DA release when using the 500Hz modulation. Additional experiments at other power levels and modulation frequencies are needed to confirm and characterize this effect.

CONCLUSIONS

Many of our results on the effects of EMFs (60 Hz @ 14 kV/m, and modulated 450 MHz fields) on the biogenic amines from rat brain have been negative. Despite some statistically significant findings in certain regions, none of the electric field-related effects was reliably found when samples were taken at only one time during the light phase of the day. However, examination of the same variables throughout the day allowed us to detect a field-related effect on the daily rhythms for NE and the main metabolite of DA. The rhythms for the serotonergic system were not significantly altered.

Exposure to 60 Hz electric and magnetic fields (up to 30 kV/m and 0.9 gauss) have been reported to alter biogenic amine metabolite concentrations in non-human primates CSF (Seegal, 1984). Using smaller electric fields in a different species, the rat, we were also able to detect alterations in the levels of neurotransmitters that could reflect either a change in the activity of central neurons or the degradatory enzymes.

It is necessary to adopt a very cautious attitude for interpretation of results from in vitro studies when examining harmful effects to intact animals or humans. One of the arguments is that there may still be no effect for in vivo studies because of the ability of the living animal to compensate or otherwise alter the responses. In our case however, in vitro experiments were generally less sensitive to the field effects than the ones obtained with in vivo exposed animals, that somewhat validates the likelihood of a biologically meaningful effect. Consequently, we feel that complete analysis of the data (now in progress) will be of great value in visualizing how these alterations affect the physiology and behavior of the whole animal throughout the day.

4.4 PROPOSED RESEARCH

METHODS

1. Apparatus

The equipment used in these experiments is described in detail in the 2.0 section (EMF Parameters and Instrumentation) of this document.

2. Procedure

Based on the results obtained to date using 60Hz electric fields, special considerations will be given to the sampling times during the day when exposing rats to combined electric and magnetic fields. As proposed in the original contract rats will receive single or repeated exposure to the fields for one hour a day, five days a week, for a period of four weeks. Three shifts of 16 animals (8 sham amd 8 exposed) will be in the exposure system at 9AM, 12PM, and 3PM respectively. In order to diminish stress related

variability in the amines, rats will be familiarized with the handling and exposure environment for one week previous to the start of treatment. Moreover, a 30 min habituation in the apparatus will precede every day session.

Animals will be sacrificed at the designated times immediately after the end of the exposure session. Brains will be removed and tissues dissected for chemical analysis as previously described. Assays of biogenic amines and opiate receptors will be performed.

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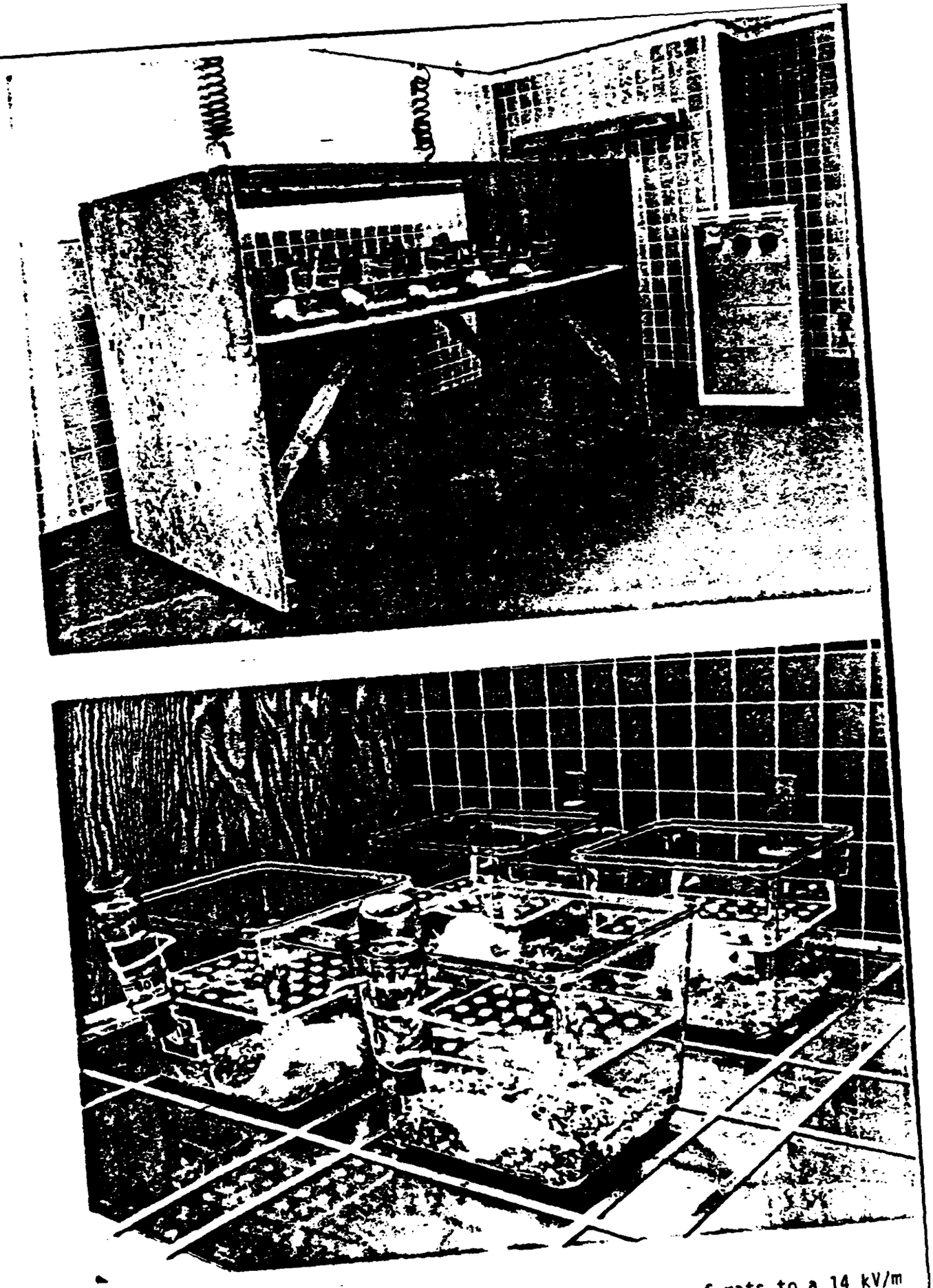

FIG. 1. Apparatus used for the continuous exposure of rats to a 14 kV/m 60 Hz electric field.

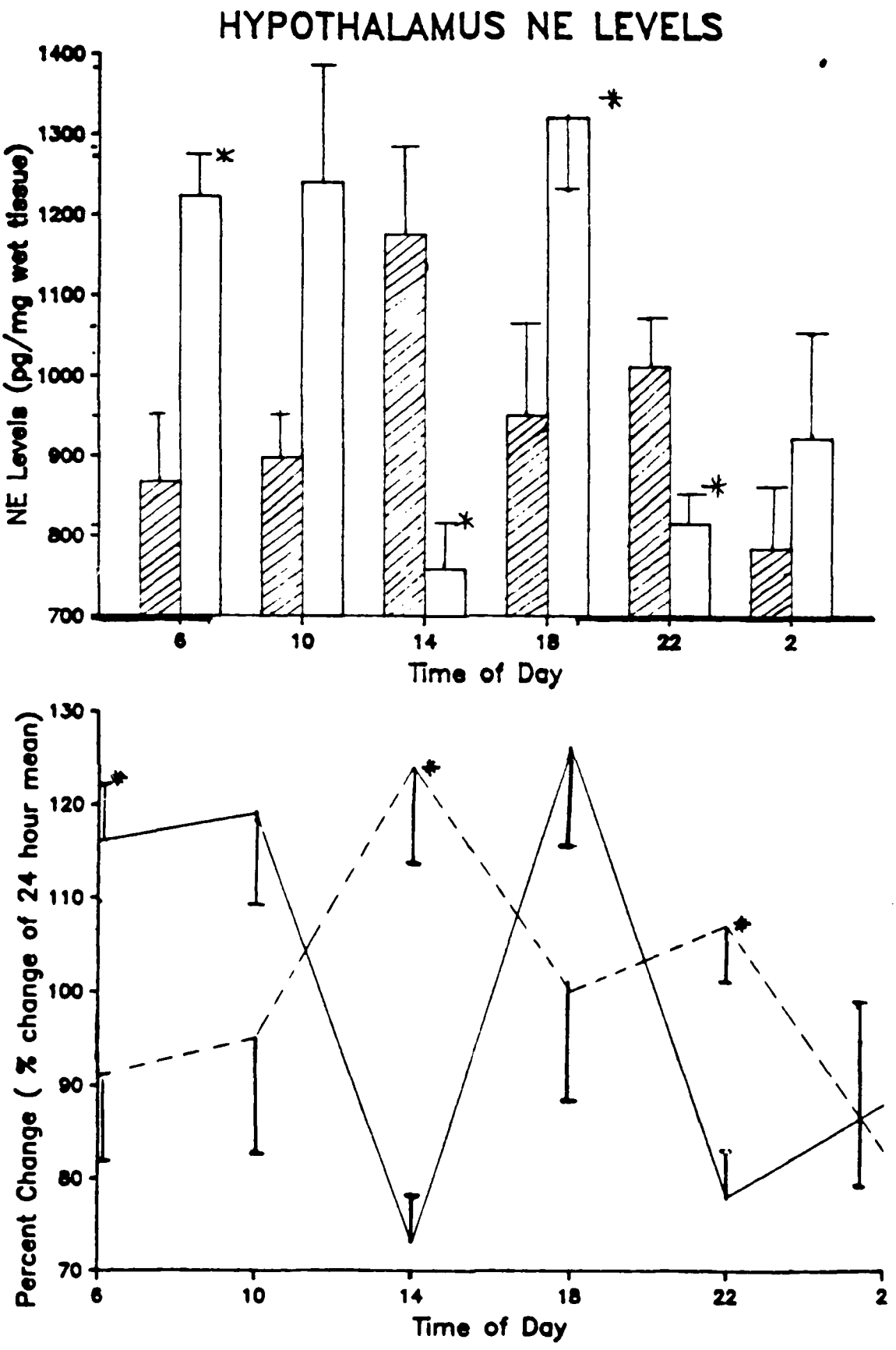

FIG. 2. Levels of NE in hypothalamus after four week exposure to a 39 kV/m, 60 Hz electric field. Top graph: Levels of NE expressed as pg/mg wet tissue. Bottom graph: Same data expressed as percent change of the 24 hr mean levels. Note the crossing over points that are responsible for highly significant interaction between type of treatment (sham or exposed) and time of day. Solid lines represent the BLUE group and correspond to open bars in top graph.

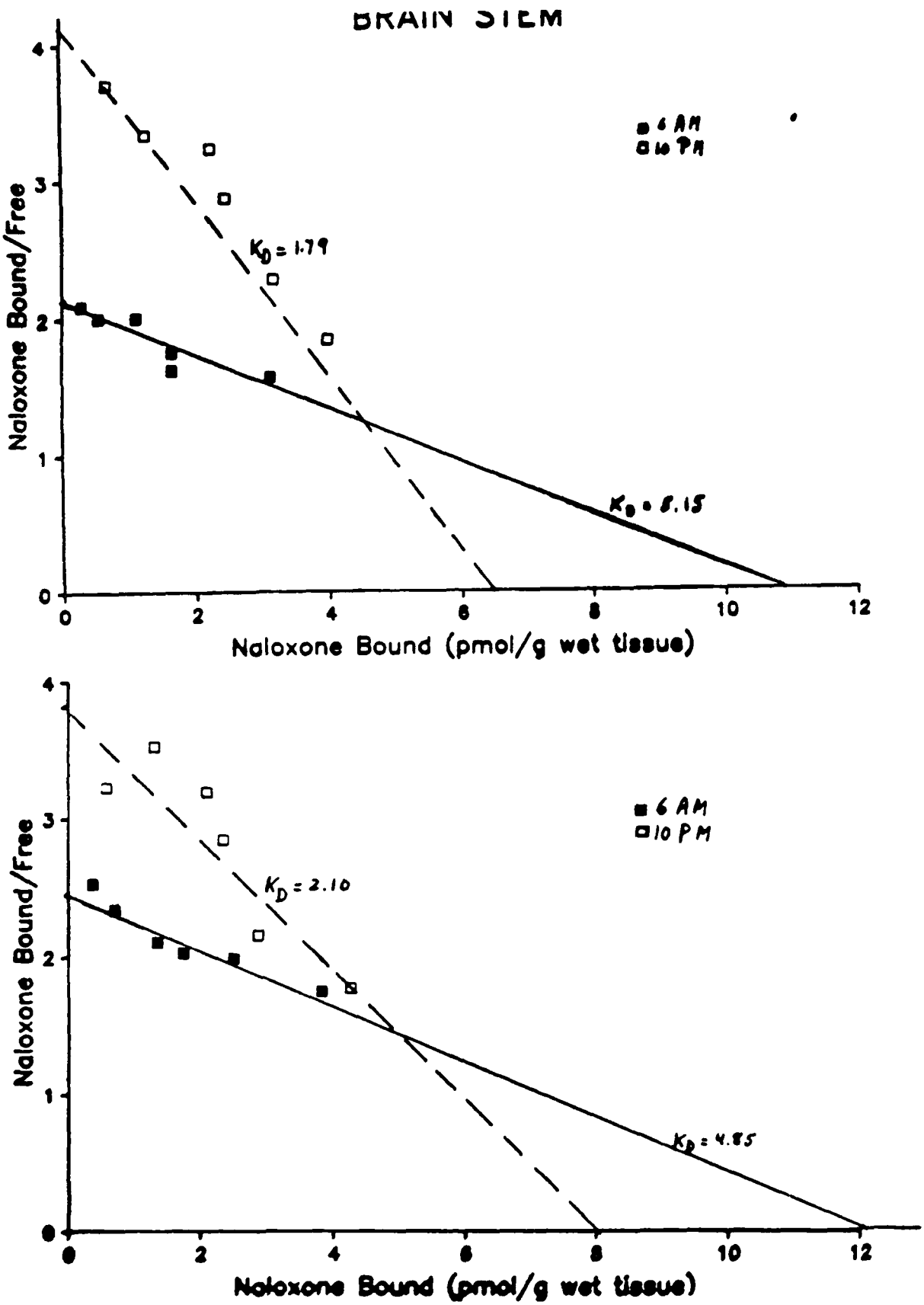

FIG. 3. Scatchard analysis of the specific binding of [^{3}H]naloxone to rat brain stem of sham and 60 Hz E-field exposed animals. Data are presented for RED (top graph) and BLUE (bottom graph) groups at two different times of day. A significant difference in affinities but not in the number of receptors was observed in both groups.

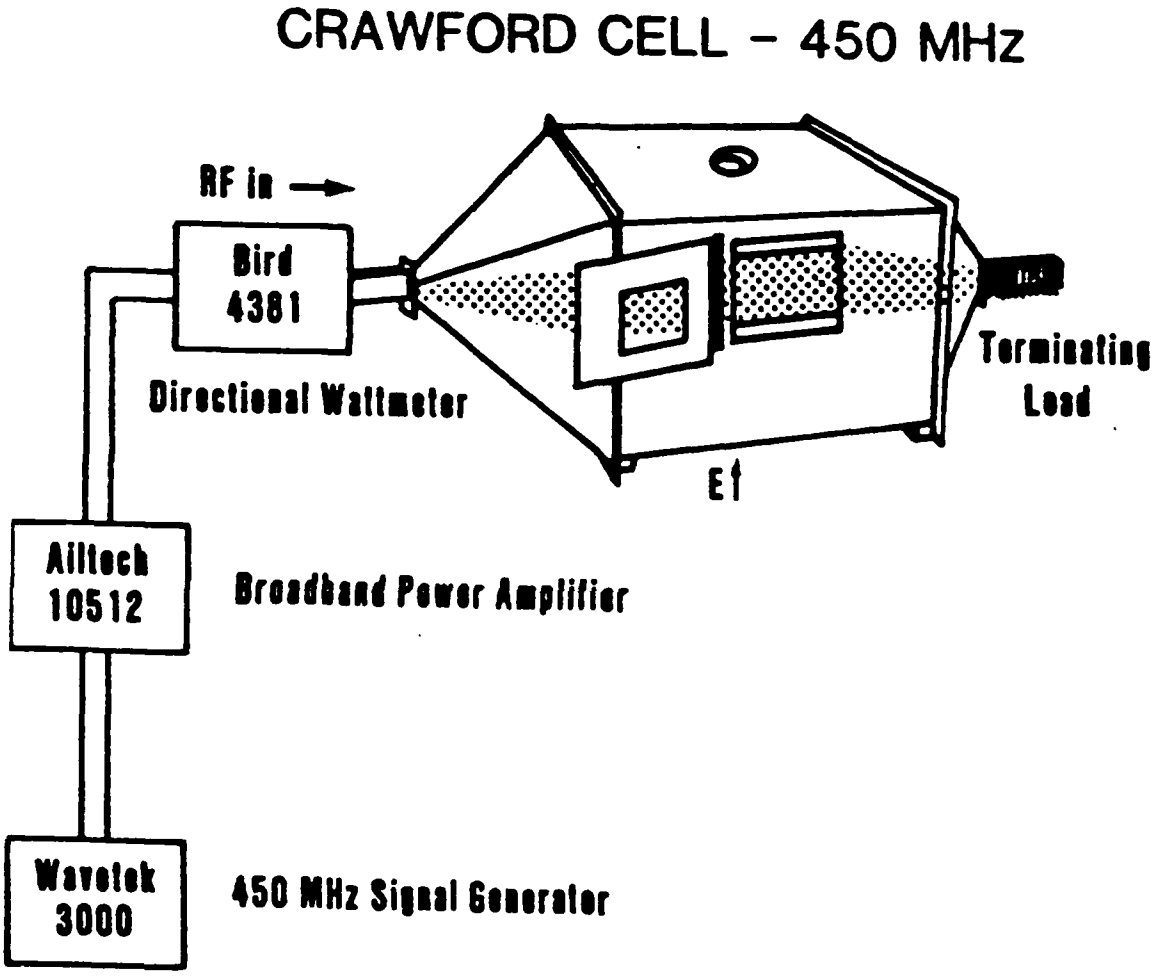

FIG. 4. TEM mode RF field exposure apparatus, model CC105S (Instrument for Industry).

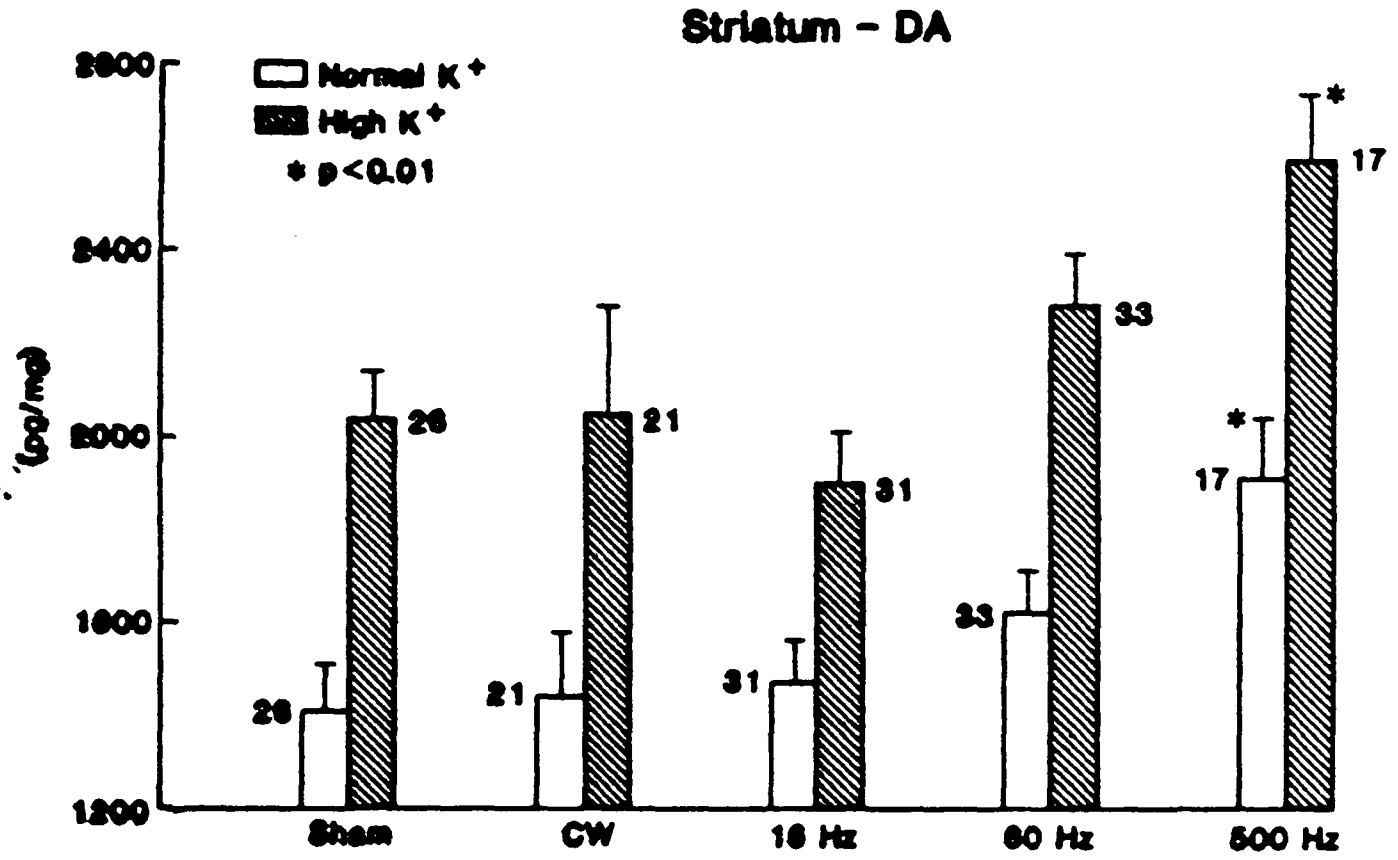

FIG. 5. Release levels of DA from rat striatum exposed to unmodulated (CW), and modulated (16, 60, and 500 Hz), low level (1.0 mW/ cm^2), 450 MHz fields in a Crawford Cell. Comparisons with sham levels showed statistically significant differences at the 500 Hz condition in both normal and high K^+ groups.

5.0 kHZ EMFs AND CELL SURFACE KINETICS: RECEPTOR MOBILITY IN EXCITABLE TISSUES. S. Lin-Liu

5.1 INTRODUCTION

The goal of this work was to study the EMF-sensitivity (pulsed fields up to 10k Hz) of the dynamic properties of cell surface macromolecules.

We have studied the possibility of field-induced molecular migration and cluster or cap formation on the surface of cultured myoblasts. Positive field effect was judged from a time-dependent reorganization of concanavalin A (con A) receptor distribution.

5.2. RATIONALE

Cell surface macromolecules play a crucial role in transducing extracellular signals across the plasma membrane. One important characteristic responsible for this function is the dynamic properties of the glycoprotein molecules. These properties have been depicted by the fluid mosaic model of Singer and Nicolson (1972) and demonstrated by the lateral movement of the glycoprotein molecules on the plane of the membrane. Physiological significance of this lateral mobility can be found in the immune system where redistribution of receptor molecules is essential for intracellular responses (Siegel et al, 1976).

Previous studies in this laboratory (Bawin and Adey, 1976, Bawin et al, 1978, Lin-Liu and Adey, 1983) and elsewhere (Blackman et al, 1982) have shown EMF sensitivity of neural tissue and suggested an interaction site at the cell surface. Externally applied DC electric fields were shown to disturb the steady state distribution of surface molecules of myoblasts and mitochondria (Poo, 1981, Zangyansky and Jard, 1979). This effect seemed to be a direct action of the DC field on charged surface macromolecules. Since the membrane surface of a nerve cell is also highly charged, we expect a similar action of the DC fields. This response may then provide a clue toward the mechanism underlying the kHz field-nerve tissue interaction. However, due to the complexity of the nerve tissue, direct observation of the membrane is extremely difficult. Since EMF was shown to interact with non-nervous tissues (Luben et al, 1982, Lyle et al, 1983) we proposed to study the action of kHz fields in a simpler system.

Single cell culture of Xenopus myoblasts was used for the following reasons: a) The undifferentiated single cells have a simple hemispherical geometry, allowing easy quantification of the receptor distribution. b) At this stage the mobility of the con A receptors was shown by Poo et al (1979) to be free from cytoskeletal control, allowing a study of the field interaction with the membrane per se. c) Myoblasts have excitable membranes, an important property shared by the neural tissue. The con A receptors were shown to be sensitive to DC electric current (Poo et al, 1979).

5.3 PROGRESS REPORT

METHODS

The experimental procedures were given in detail in the original proposal and reported in a publication in Biophys.J. (Lin-Liu et al, 1984, attached). To summarize, cells were first exposed to electric current in a small chamber with defined geometry. They were labeled with fluorescence- conjugated con A which binds to the sugar moiety, glucose and manose, of surface glycoproteins. The fluorescence image of the cells was then subjected to examination and measurement under a fluorescence microscope. With our labeling technique, the fluorescence staining on cell surface marked the location of con A receptors. To assess receptor cluster and cap formation, the pattern of the staining was examined visually and recorded on film. For the migration studies, fluorescence intensities at the two poles of the spherical cells facing the cathode and the anode of the applied field were measured with the method of microfluorimetry modified from Poo et al (1979). Since we are concerned only with the relative fluorescence intensities over the surface of each cell, the intensity difference at the two poles was normalized against the sum of intensities to obtain an asymmetry index (AI). Pre- and post-field asymmetry indexes were compared statistically to asses possible field-induced molecular migration.

Monopolar pulsed electric fields of frequency 0.5-10.0 kHz, intensity range 0.1-3.8 V/cm were used. Duty cycle ranged between 15 and 75%. Bipolar asymmetric fields with zero net current were also used.

RESULTS

The most significant finding of this study was that monopolar pulsed electric fields induced con A receptor redistribution on myoblast cell surfaces and the effectiveness of the field was dependent on the duty cycle of the pulses. Part of the results have been published (Lin-Liu et al, 1984). The following is a summary.

5.3.1 Lack of con A Receptor Aggregates

Since molecular aggregate formation can cause significant signal transduction in the cells as in the immune system (Siegel et al, 1976), we examined the possibility of receptor aggregate formation in the fields. Under all experimental conditions used, the fluorescence staining exhibited no pattern of cluster or capping on the cell surfaces. This indicates that the con A receptors in our system do not form macroscopic aggregates with or without electric field exposure (Fig. 1, left and right). Whether aggregates formed from small number of receptor molecules under any of the experimental condition is beyond the detection limit of the present study.

5.3.2 Field-induced Asymmetry of con A Receptor Distribution

The uniform pre-field distribution of con A receptors (Fig.1, left) became polarized following exposure of the cells to most of the fields used in our studies. An example of field-induced polarization is shown on the right in

Fig.1. The extent of polarization (asymmetry) depended on the duration of the field, pulse width, frequency and intensity. This apparently complex relationship was greatly simplified by expressing the results in function of the duty cycle of the applied fields, a parameter determined by both pulse width and repetition rate.

a) DC electric fields

DC field exposure was performed to provide comparison with pulsed field exposure. Our experiment confirmed the results published by Poo et al. (1979) that DC field exposure resulted in a higher con A receptor concentration on the area of the membrane facing the cathode. This effect was detectable following 5 min exposure (Fig. 2) reaching an apparent plateau in about 30 min and it was reversible after field removal (not shown here). This reversibility was the result of diffusional randomization of the con A receptors.

b) Monopolar pulsed electric fields

Pulsed electric fields of 50% duty cycle caused con A receptors to redistribute on the cell surface in a way similar to that induced by DC fields. The time courses of asymmetry development (from 5 to 30 min) were similar for pulsed fields and DC fields of the same polarity and equivalent average intensity (see Fig.2). Pulses of frequency between 0.5 and 10 k Hz produced similar results (see Fig. 3), suggesting that repetitive diffusional randomization of the molecule during the interpulse intervals did not influence the time course of asymmetry development.

Longer exposures were required to induce polarization with pulsed fields of low duty cycle. For example, 5 ms pulses in a 10 Hz field of 6 V/cm produced detectable asymmetry within 150 min of exposure. This corresponds to a field of 5% duty cycle and an average intensity of 0.3 V/cm within each cycle. A higher frequency field was required for a more rapid induction of asymmetry with 5 ms pulses. Thus, 100 Hz field (50% duty cycle) and lower pulse intensity (3V/cm, average 1.5 V/cm) produced significant asymmetry within 5 min (see Fig.2).

A direct comparison on effectiveness was made between the DC fields and the pulsed fields of duty cycles varying from 15% to 75% (Fig. 4). A ratio value of 1 on the ordinate indicates equal effectiveness for pulsed and equivalent DC fields. Interestingly, significant differences were found for 5 min exposure, but not for 10 min exposure when the field effects had reached a plateau (steady state). In 5 min, lower duty cycle fields produce less asymmetry than DC field, while the reverse effect was seen with high duty cycle fields. This comparison suggested a critical value of the duty cycle at around 25% for pre-steady state. For duty cycles below this value, pulses were less effective than DC fields or pulses of duty cycle above 50%.

c) Bipolar Asymmetrical electric fields

The results reported above demonstrated that for equal amount of charge per pulse, wide pulses (e.g., 75% duty cycle) are more effective than narrow pulses (e.g., 25% duty cycle). It is interesting to point out that the local

extracellular electric fields generated by a neural action potential resemble a bipolar asymmetric field condition with pulses of opposite polarities representing for example 10 and 90% of the cycle time, with an intensity ratio of 9:1. In these conditions, the overall current generated during each cycle is zero. No induced asymmetry would be expected with prolonged exposure to such a field, since the average field intensity is zero. Attempts to explore asymmetry following brief exposures to bipolar fields were unsuccessful because the effect was too small to measure reliably (Fig.5).

CONCLUSIONS

The present experiment demonstrated the effectiveness of the pulsed fields in producing reorganization of receptors on cell surfaces. This effect can be considered separately in two phases; the steady state and the pre-steady state. In the steady state condition, pulsed fields of different patterns but the same average field intensity produce the same effect, which is also identical to that produced by equivalent DC fields. In the pre-steady state, the lower duty cycle field is less effective than the higher duty cycle field of the same average field intensity, as well as the equivalent DC field. Frequency dependence in pre-steady state is implied by these factors.

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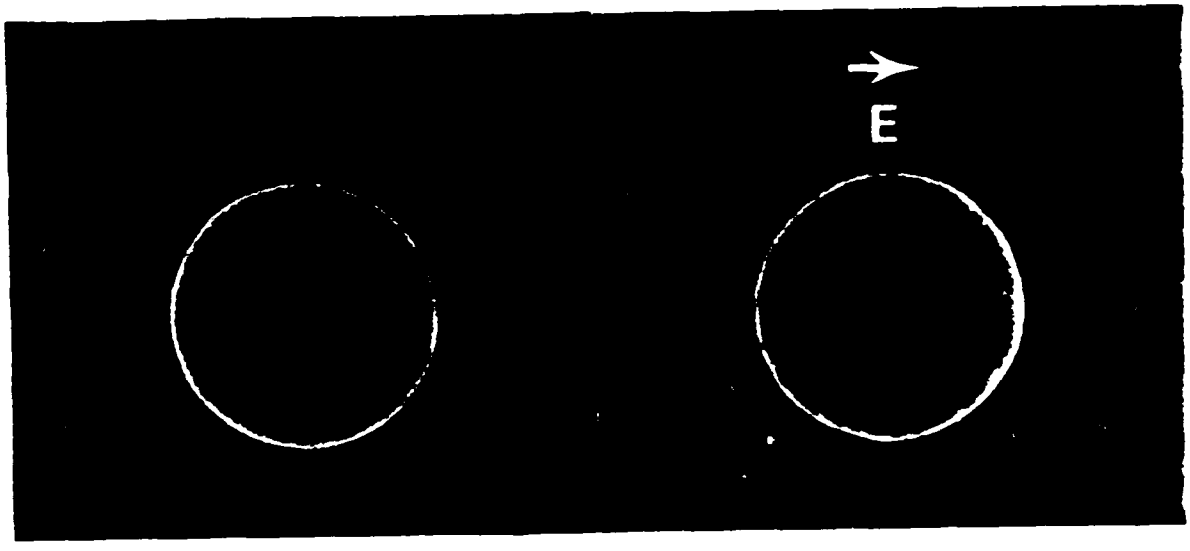

FIGURE 1. Fluorescence labeling of myoblast cell surface con A receptors for a no field (left) and a pulsed field exposure (right) conditions. Fluorescence staining was rather smooth over the cell periphery with no obvious clusters or capping in either case. Following field exposure, the fluorescence intensity was polarized toward the cathode.

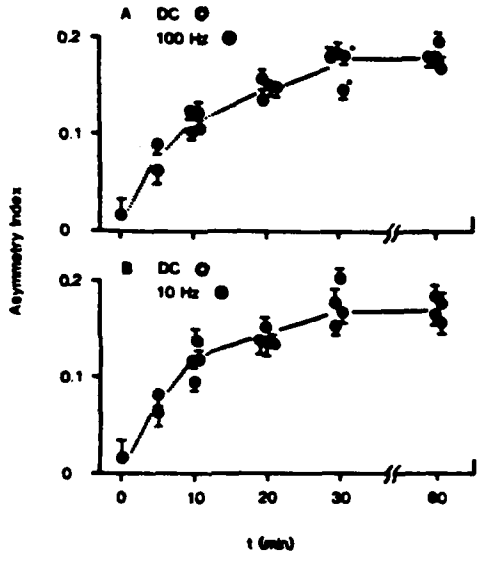

FIGURE 1 Time course for the development of asymmetry in con A receptor distribution after exposure to DC fields and 50% duty cycle pulses. Pulse intensity was always 3 V/cm and DC field intensity was 1.5 V/cm. Data show comparison between DC and (*A*) 100 Hz and (*B*) 10 Hz pulsed fields. Each data point (mean ± SEM) was determined from 22–28 cells in one culture. Each pulse-exposed culture was accompanied by a simultaneous DC exposure to a parallel culture. At each time point, these paired data were plotted with error bars pointing toward the same direction. Statistical analysis (Student's *t*-test) of the pairs showed no significant difference between the results of DC and pulsed field experiments ($P > 0.05$) except the case of marked * where $0.01 < P < 0.05$.

FIGURE 2 (From Lin-Liu et al., 1984)

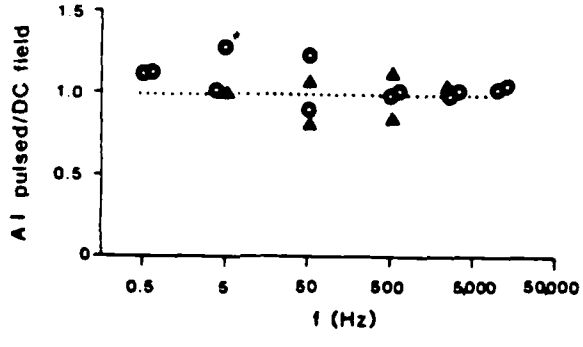

FIGURE 6 Effectiveness of 50% duty cycle pulses over an extended frequency range (0.5 Hz to 10 kHz). Each point represents the ratio obtained similar to those of Fig. 5. Horizontal dotted line indicates results from DC field exposure. DC field intensity was 1 and 1.9 V/cm for pulses 2 (▲) and 3.8 (○) V/cm, respectively. Exposure duration was 20 min. Comparison between DC and pulsed field exposure showed no statistically significant difference at 95% confidence level ($P > 0.05$) except for the case marked with * to its upper right, where $0.01 < P < 0.05$.

FIGURE 3 (From Lin-Liu et al., 1984)

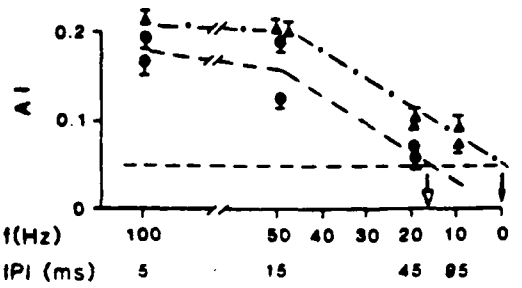

FIGURE 2 Dependence of asymmetry index on interpulse interval (IPI) or frequency of pulsed fields with fixed pulse width (5 ms), intensity (6 V/cm) and total number of pulses delivered. Pulses were delivered at various frequencies to achieve different IPIs. Although the time-averaged field intensity was lower at longer IPI, the total amount of charge passed remained the same. Within each group of experiments (6×10^4 [●] or 9×10^4 [▲] pulses), all 20 and/or 10 Hz results are statistically different from 100 and 50 Hz results ($P < 0.01$, Student's t test). Each point (mean ± SEM) was obtained from 22–28 cells in one culture. Asymmetry index of 0.05 indicates level of detectable asymmetry (straight dotted line). For 6×10^4 pulses (●), a threshold frequency between 10 and 20 Hz (,) (IPI 50–70 ms) was obtained by extrapolation. For 9×10^4 pulses (▲), detectable asymmetry may be achieved at frequency below 10 Hz (,).

FIG. 4a

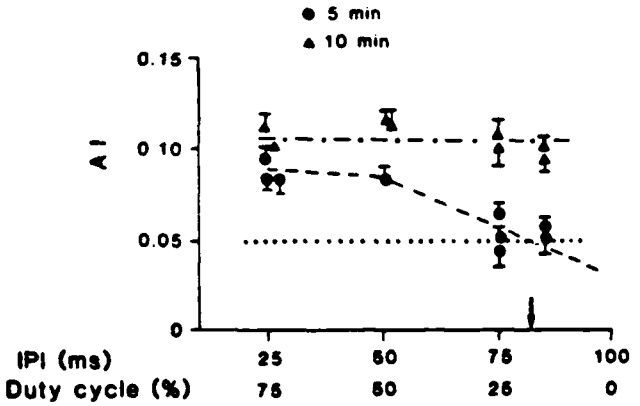

FIGURE 3 Dependence of asymmetry index on interpulse interval (IPI) of pulsed field with fixed frequency (10 Hz). Short term exposure (5 and 10 min) was examined. To keep an average field intensity of 1.5 V/cm for all IPI values used, pulse intensity was varied and was 10, 6, 3, and 2 V/cm for IPI 85, 75, 50, and 25 ms, respectively. At 5 min, all results from IPI ≥ 75 ms are statistically different from IPI = 25 ms ($P < 0.01$, Student's t test). At 10 min, the same extent of asymmetry was achieved by all field conditions. Each point (mean ± SEM) was obtained from 22–29 cells in one culture.

FIG. 4b

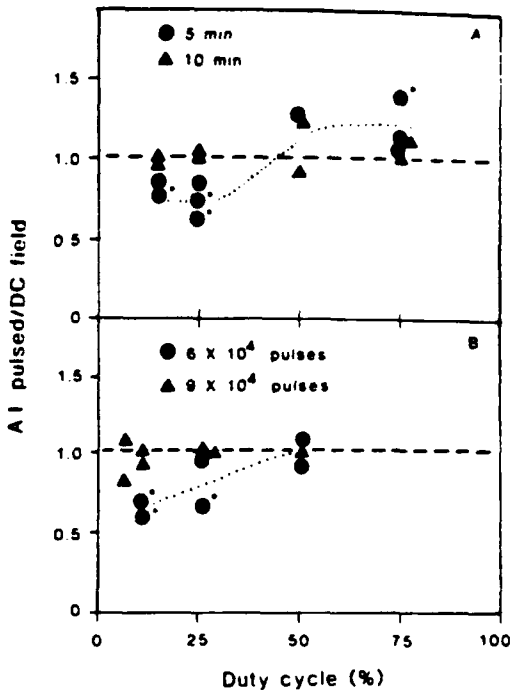

FIGURE 5 (*A*) Effectiveness of 10 Hz pulsed fields with various duty cycles. Each point represents the ratio of two mean *AI* (22–28 cells each) determined from two parallel cultures, one exposed to pulsed and the other to DC field. The pulsed field conditions were the same as those of Fig. 3. DC field intensity was 1.5 V/cm. (*B*) Effectiveness of 5 ms pulses delivered as fields of various duty cycles. Each point represent the ratio obtained similar to those of *A*. Pulsed field conditions were the same as those in Fig. 2. DC field intensity corresponded to the average field intensity of the pulsed field and was 0.3, 0.6, 1.5, and 3 V/cm for duty cycle 5, 10, 25, and 50%. In both *A* and *B*, horizontal dotted line indicates results from DC exposure in the corresponding conditions. Student's t test on paired results (before taking ratio) from DC and pulsed fields showed statistically significant differences at 95% confidence level ($P < 0.05$) for points marked with * to their *upper right*.

FIG. 4c

FIGURE 4 (From Lin-Liu et al., 1984)

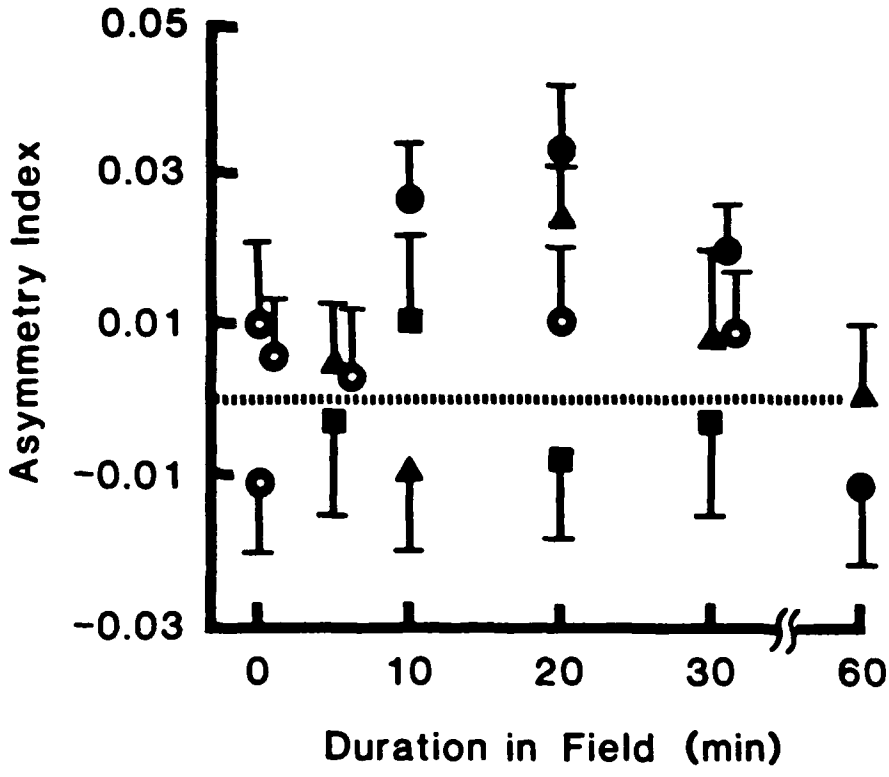

FIGURE 5. Asymmetry index following exposure to asymmetric fields composed of two pulses of opposite polarities in each cycle. The widths and intensities of the two pulses were adjusted to deliver the same amount of charges and generated zero net current for each cycle. Each data point was the mean and SD from at least 20 cell measurements. All data points are within 2 S.D. distance from zero, indicating no detectable asymmetry formation. Ratios of pulse height in the two polarities were ○ 50/50, ▲ 75/25, ■ 80/20, ● 90/10. Frequency was 100 Hz.

6.0 EFFECTS OF kHz FIELDS ON ORNITHINE DECARBOXYLASE ACTIVITY IN VITRO

C.D. Cain

6.1 INTRODUCTION

A primary site of interaction of low-frequency nonionizing electromagnetic radiation with tissue is at the cell membrane. Navigational and communication systems of low frequency radio spectrum from 10 to 100 kHz fields produce current densities and electric field strengths in tissue that are 5 - 6 orders of magnitude lower than current densities associated with membrane depolarization and electric field strengths across the cell membrane. To record the effects of these interactions, the external events on the cell surface must be amplified to measurable parameters. Fortunately, enzymatic systems within the cell membrane offer tremendous amplification of external signals on the cell surface.

6.2 RATIONALE

Adenylate cyclase and phosphatidylinositol metabolism are two primary membrane-associated systems that transduce signals across the cell membrane. The result is the production of second messengers, cyclic adenosine monophosphate (cAMP) by adenylate cyclase, inositol triphosphate (IP3) and intracellular Ca++ by phosphatidylinositol metabolism. We (Luben et. al., 1982, Luben and Cain, 1984, Cain and Luben, 1985, and Cain et. al., 1985) and others (Murray and Farndale, 1985, and Farndale and Murray, 1985) have demostrated that weak electromagnetic fields influence cAMP metabolism in cell culture systems. Thus, second messengers are transiently (within seconds) the first biochemical markers that measure field-affected transductive processes across the cell membrane.

Subsequent to second messenger production, the cell further amplifies its response to external signals by activation of numerous enzymatic pathways. For example, protein kinases are activated by the above second messengers within minutes of external stimulation. cAMP-independent protein kinases have proven to be sensitive to weak amplitude-modulated microwave fields (Byus et. al. 1984).

Ornithine decarboxylase (ODC) activity responds to external signals through both the adenylate cyclase cascade and the phosphatidylinositol cascade. This enzyme is highly regulated and its activity can be increased up to 1000-fold by external signals, such as hormones and growth factors. Thus ODC activity is an excellent enzymatic marker to monitor membrane signal amplification. Therefore ODC activity is a prime candidate to indicate field interactions at the cell membrane.

ODC's importance in cellular growth and proliferation is another reason for its appropriateness as a measure of field interaction. ODC is the rate limiting enzyme in polyamine biosynthesis and is absolutely required for cellular growth in all eukaryotic cells. Therefore, field effects on ODC activity can be indicative of changes in cell growth and proliferation.

6.3 PROPOSED RESEARCH

METHODS

6.3.1 Exposure System

The agar-bridge system was designed to expose cells in tissue culture to extremely low frequency (ELF) fields in such a manner that field strengths (0.1-10 mV/cm) and current densities (2.0-200 uA/cm2) are fairly uniform, well defined, and measurable in the tissue culture medium. In addition, the agar-bridge system allows biochemical assays to be done quickly and efficiently. In series, current is passed through growth medium in petri dishes (9 cm x 9 cm) and petri dishes are connected by glass bridges that are filled with growth medium and 1% agar. This design allows for uniform field exposure to different petri dishes. After exposure, we will treat the cells with various concentrations of hormones and tumor promoters.

The composition of a glass bridge includes two concentric half-circular glass tubes that have different diameters (28 mm and 41 mm) so that 1% agar solution (10 ml) can be poured between the tubes. The tubes are 8 cm long and are sealed at the ends. The resistance of a bridge is 240 ohms which is comparable to the resistance across a petri dish with medium, 170 ohms. The electric field is generated by a constant current amplifier and Wavetek sine-wave generator (See description of exposure system in section 2.0 of this document-ELF Field Parameters and Instrumentation).

6.3.2 Ornithine Decarboxylase Assay

Since ODC activity in primary bone cells is sensitive to low-frequency pulsed electromagnetic fields (Cain et al., 1985), we will test the effects of kHz fields using the same technique. Other studies have indicated that modulated microwaves affect ODC activity in Reuber H35 hepatoma cells (Byus et. al., 1985).

Cells are grown in minimum essential medium (MEM) with 10% fetal bovine serum (FBS) in 9 x 9 cm petri dishes. After cells have reached confluency for 2 - 3 days, they are ready for field exposure in the agar-bridge system. After field exposure, ODC activity is determined according to the method of Russell and Synder (1969). The cells are washed twice with ice cold phosphate buffered saline (PBS) and scrapped off. The cells are spun in a clinical centrifuge and the cell pellet is sonicated in an ODC assay mixture (50 mM sodium-potassium phosphate pH 7.2, 1 mM dithiothreitol (DTT), 0.1 mM ethylene-diamine-tetra-acetate (EDTA), 0.1 mM pyridoxalphosphate (PLP)) and assayed in a total volume of 0.2 ml. ODC activity is determined by measuring the amount of $^{14}CO2$ released from 0.5 uCi of L-1-^{14}C-ornithine (Amersham, 59 mCi/mmole) plus sufficient unlabelled ornithine to bring the total substrate concentration to 0.25 mM during a 60-minute incubation at 37°C. Enzymatically released $^{14}CO2$ is trapped in a special center well containing ethanolamine-ethoxyethanol (2:1) fitted over a 15 ml conical centrifuge

tube. Following the 60-minute incubation, the enzyme reaction is stopped by injection of 0.5 ml of 2 M citric acid directly into the reaction mixture. After overnight incubation, at room temperature, the center well is removed and the radioactivity measured in 10 ml of aqueous scintillation fluid. One enzyme unit of ODC is defined as the amount of enzyme required to catalyze the release of 1 nanomole of CO2 from L-ornithine in 60 minutes.

6.4 REFERENCES

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CELLULAR AND ORGANISMAL RESPONSES TO COMBINED KILOHERTZ AND OTHER NONIONIZING ELECTROMAGNETIC FIELDS.

RESEARCH ACTIVITIES FOR PERIOD APRIL 1986 - NOVEMBER 1987

W.R. Adey, M.D.
S.M. Bawin, Ph.D.
C.D. Cain, Ph.D.
A.R. Sheppard. Ph.D.
M.E. Stell, Ph.D.
B.J. Vasquez, Ph.D.

EFFECTS OF EXPOSURE TO A 100 kHz LORAN-C TYPE WAVEFORM ON THE RAT BRAIN NEUROTRANSMITTER SYSTEMS. B.J. Vasquez, S.M. Bawin, and W.R. Adey.

INTRODUCTION

The goal of this research was to examine possible biological effects of exposure to the near-field of Loran-C transmitters that operate in a pulse-modulated mode at a carrier frequency of 100 kHz. Experiments were designed to study the effects of single and repeated exposure to a simulated Loran-C type waveform on the rat brain biogenic amine and opiate receptor systems. These systems are involved in the endocrine control of biological rhythms and responses to environmental stresses.

METHODS AND PROCEDURES

I. EXPOSURE SYSTEM

Exposure apparatus and field measurements have been completely described in previous reports (Final & Request for Extension Report (1-14-86) and Annual Report (7-29-86).

II. PROCEDURE

1. Habituation: Animals were handled and placed into the exposure apparatus for three consecutive days.

2. Exposure schedule:

   a) Animals were placed in the apparatus at 9:00 h (AM group) or 15:00 h (PM group).

   b) 30 min habituation.

   c) Field or sham exposure for 1 hr per day, five days a week, for up to 4 weeks.

   d) Number of exposures : 1, 5, and 20.

3. Sampling: Animals were immediately sacrificed at the end of their exposure period. Brains were dissected into 6 regions: striatum, hypothalamus, hippocampus, anterior and posterior cortices, and brain stem. Adrenal glands were also collected.

4. Biogenic amines and acidic metabolites were measured by HPLC-ED.

5. Opiate receptors were measured by binding assays using 3H-naloxone and levallorphan.

6. Data were analysed by two-way analysis of variance (ANOVA) and "a posteriori" group comparisons by Newman-Keuls tests.

RESULTS

1. The first experiment (March 1986) showed no field effects after 1, 5, or 20, 1 hr-exposures. We then performed two replications (July and October 1986) using a 4-week exposure period (twenty 1 hr-exposures) to facilitate comparison between these results and our previous work with 60 Hz electric fields (Vasquez et al., Bioelectromagnetics, 1988, 9: 229-236).

2. In the hypothalamus, we found significant AM-PM differences in the biogenic amine levels across the three replications, but no field related effect after either single or repeated exposure. There were no consistent differences between the AM and PM levels of biogenic amines in striatum, cortex, and hippocampus. Significant field-related changes were observed for most of the amines in the cortex only during the July replication.

3. Levels of catecholamines in the adrenal glands were not affected by field exposure indicating the absence of generalized stress.

4. Number and affinity of opiate receptors in the posterior cortex and brain stem did not show any field-related changes at the times sampled.

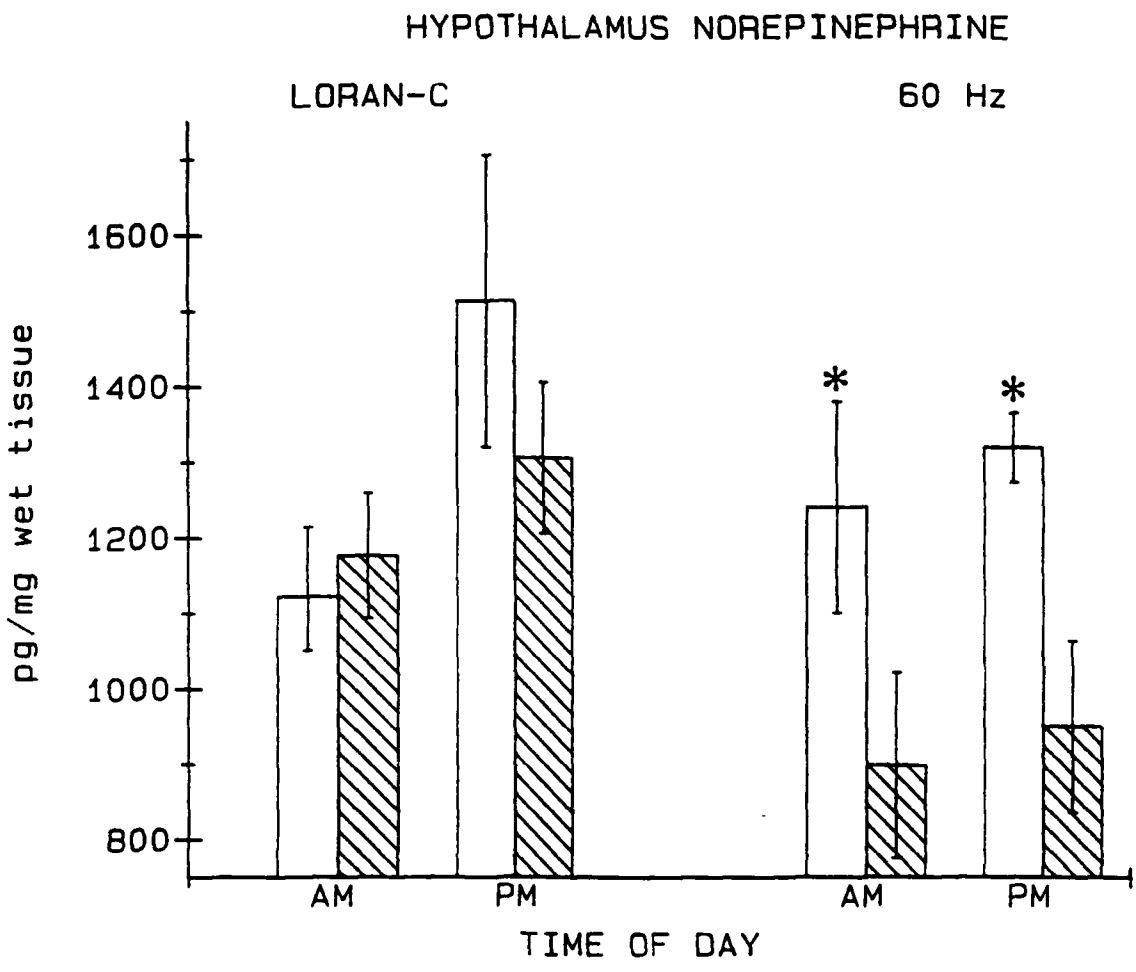

FIG. 1 : Levels of norepinephrine in the rat hypothalamus after 4-week exposure to 100 kHz combined electric and magnetic fields (left panel) and 60 Hz electric fields (right panel). Hatched bars represent data from field exposed groups. Although AM-PM differences in levels were observed in both experiments, the field effect was only seen at comparable times after exposure to 60 Hz Hz electric fields. Note that sampling was obtained at 4 hour=intervals over a 24 h period following exposure to 60 Hz electric fields. These studies suggested a shifting in the normal daily rhythms of the amine, which could not be observed with the single AM and PM measurements performed here.

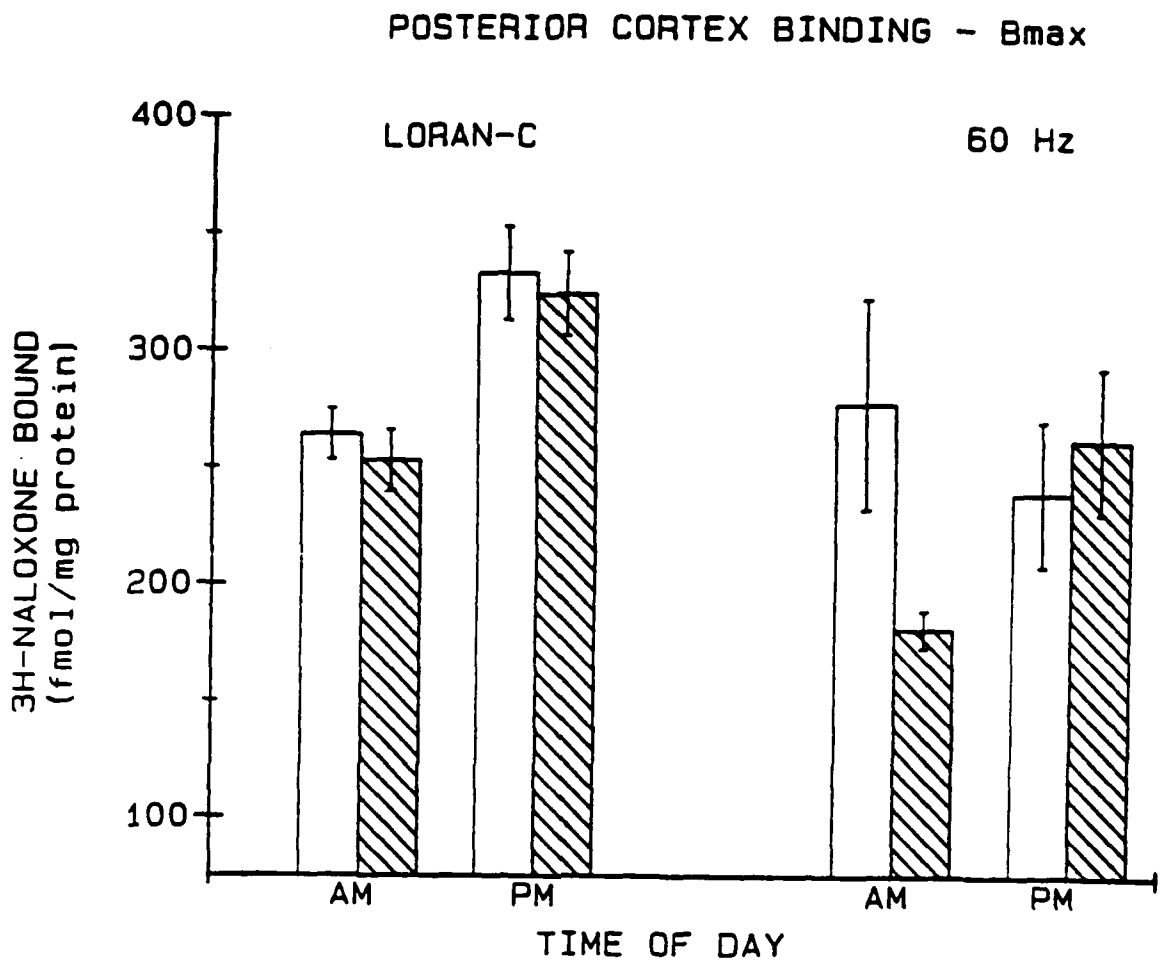

FIG. 2: Number of opiate receptors in the rat posterior cortex of rats after 4-week exposure to 100 kHz combined electric and magnetic fields (left panel) and 60 Hz electric fields (right panel). Hatched bars represent data from field exposed groups. In both experiments we found diurnal changes but no field-related effect. However, the 60 Hz study included samples collected troughout the day and showed that electric fields can alter the daily rhythms of the receptor system resulting in significant differences between groups at several times of the day.

CONCLUSIONS

Loran-C type fields did not induce consistent changes in the rat brain biogenic amine and opiate receptor systems when applied once a day for up to 4 weeks. However, we can not conclude that another regimen of exposure would not affect either system since we were able to detect 60 Hz field-induced changes in the diurnal rhythms of biogenic amines and opiate receptors when sampling every 4 h throughout the day.

SENSORY PROPERTIES OF A 100 kHz LORAN-C TYPE WAVEFORM. M.E. Stell and W.R. Adey.

INTRODUCTION

The purpose of this study was to determine if rats could give behavioral evidence of detecting the Loran-C type waveform. The experimental paradigm was that used by Sagan et al. (Bioelectromagnetics 8:303-313, 1987). In the initial study summarized in the Final & Request for Extension Report (1-14-86) two subjects were trained and tested. These subjects gave no evidence of being able to detect the field. This lack of detection led to two variations of the protocol described below.

Both variations attempted to maximize the sensitivity of the experimental paradigm. The first paradigm trained subjects on a closely related task, detection of a 60-Hz electric field. Two subjects were trained to detect 60-Hz field and then tested for detection of the 100-kHz Loran-C type field. The purpose was to allow the rats to gain experience with the paradigm and with what is presumably the most directly comparable sensory stimulus.

The second variation used the Sagan et al. paradigm, but placed extreme emphasis on maximum preparation at each stage of the protocol. It was hoped that if the 100-kHz field detection was possible, albeit difficult, that careful training of the subjects would maximize their ability to detect the fields.

METHODS AND PROCEDURES

I. EXPOSURE SYSTEM

Exposure apparatus and field measurements have been completely described in previous reports (Final & Request for Extension Report (1-14-86).

II. PROCEDURE

1. The general procedures are described in the accompanying report. For the first variation (detecting 60-Hz electric fields before 100-kHz fields) 2 rats were again trained to detect a tone, as in the original protocol. Next, instead of fading out the tone while introducing the 100-kHz field, the tone was faded out while introducing a 60-Hz electric field. As demonstrated previously, these subjects had no difficulty detecting this electric field. After sufficient training, when the rats were reliably and successfully detecting this field, the 60-Hz field was replaced by the 100-kHz field. Everything else in the paradigm was kept as identical to the

60-Hz detection experiment as possible. The subjects were immediately moved from the 60-Hz test chamber to the 100-kHz test chamber. The behavioral response requirements were identical (the same control software was used), the size and materials in the test chamber were identical, and the parallel plate electrode system was an exact replica of the 60-Hz field system.

2. For the second variation (maximal training at each stage of the protocol) the original protocol was used, but this time each stage was extended to allow the subjects the maximum experience and training before going on to the next stage.

RESULTS

1. Results of the first variation showed that the subjects learned to successfully detect the tone and the 60-Hz electric field as expected. When the 60-Hz field was removed and the 100-kHz field was used the subjects ability to detect the electric field disappeared. The switch from 60-Hz to 100-kHz was made by simply placing the subjects in the 100-kHz exposure test chamber, so the transition between fields was quite sharp. The performance of the subjects changed in a similar (quite sharp) fashion. The two subjects had been reliably and successfully detecting 60-Hz fields, but from the first day did not demonstrate any ability to detect 100-kHz fields. Continued training of these subjects with the 100-kHz field did not change their complete lack of ability to detect this stimulus.

2. The results of the second variation were that the subjects learned the detection task well up to and including detection of the tones. After considerably more tone detection than is normally given to rats in the 60-Hz detection paradigm, the tone was faded out and the 100-kHz field replaced it. As was the case in previous 100-kHz sessions, as the tone faded out, the subjects performance became closer and closer to random responding. When the tone was removed, the subjects performance became completely random, with no evidence of any ability to detect the fields.

CONCLUSIONS

The results of the original 2 subjects were replicated in 6 more subjects, using two variations of the original protocol. Although the number of subjects is small, each subject was tested for his ability to detect the field many thousands of times. This lends considerable confidence to the conclusion that rats cannot detect 100-kHz electric fields as used in this experiment.

The possibility exists that 100-kHz fields may be detectable if presented at higher intensities or with a greater duty cycle. However, the purpose of our research was to determine if fields similar to the 100-kHz Loran-C field reported by McEnroe in 1980 were detectable in an established laboratory protocol. This experiment suggests that they are not.

EFFECTS OF kHz FIELDS ON ORNITHINE DECARBOXYLASE ACTIVITY IN VITRO. C.D. Cain, A.R. Sheppard, and W.R. Adey.

INTRODUCTION

A primary site of interaction of low-frequency nonionizing electromagnetic radiation with tissue is at the cell membrane. Navigational and communication systems of low frequency radio spectrum from 10 to 100 kHz fields produce current densities and electric fields strengths in tissue that are 5 - 6 orders of magnitude lower than current densities associated with membrane depolarization and electric strengths across the cell membrane. To record the effects of these interactions, the external events on the cell surface must be amplified to measurable parameters. Fortunately, enzymatic systems within the cell membrane offer amplification of external signals on the cell surface.

Ornithine decarboxylase (ODC) is a highly regulated enzyme and its activity can be controlled by external signals such as hormones and growth factors. Therefore, its activity is an excellent marker to monitor the consequences of influences on membrane signal transduction.

METHODS AND PROCEDURES

Exposure system and assay procedures have been completely described in previous reports (Final & Request for Extension Report (1-14-86) and Annual Report (7-29-86)).

RESULTS

Since we found that ODC activity in primary bone cells is sensitive to low-frequency pulsed electromagnetic fields (Cain et al. 1985), we have tested the effects of a 60-Hz electric field on the same preparation. The agar-bridge exposure used was described in the Annual Report, July 1986. This system can deliver fields of similar magnitude to the endogenously generated current densities at the bone surface. The average of 4 experiments showed that a 1-h exposure to a 10 mV/cm field with an associated current density of 160 uA/cm^2 increased ODC activity two-fold when assayed 10 min to 1 h after field exposure. At 2- and 3-h post-exposure, ODC activity returned to sham exposed levels.

Our next step was to examine if the Loran C waveform at 10 mV/cm strength affected ODC activity in these cells. Under the same protocol used with the 60-Hz field, the Loran-C field did not

modify basal ODC activity in primary bone cells.

As observed in primary bone cells, the 60 Hz field, 10 mV/cm, also increased ODC activity in mouse fibroblasts (C3H10T1/2). In four experiments, the field increased activity up to two-fold after 1-4 h exposures. This effect persisted up to 4 h after cessation of the field. Under the same protocol, the Loran-C field did not increase the ODC activity in the fibroblasts.

CONCLUSION

Under similar protocols, in which pulsed electromagnetic fields and 60-Hz electric fields were able to influence ornithine decarboxylase activity in primary bone cells and fibroblasts, the Loran-C waveform did not affect ornithine decarboxylase activity.